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TWO-YEAR PERFORMANCE OF TWO DISEASE-TOLERANT OYSTER STRAINS IN THE PATUXENT RIVER, MARYLAND

George R. Abbe (Morgan State University), Carol B. Mccollough (Maryland Department of Natural Resources), Christopher F. Dungan (Maryland Department of Natural Resources)

Extreme mortalities among a standard strain of specific-pathogen-free (SPF) *C. virginica* at three sites in the Patuxent River, Maryland during the 2000-02 drought, led to our search for improved performances from available disease resistant native oyster strains. SPF spat of disease-tolerant CROSBreed and DEBY strains, alongside standard-strain oysters, were set and deployed in September 2003, at our same experimental sites. Mean station salinities ranged from 12.1-16.2 ppt during 2000-02, but have been generally lower since then, although salinity increases were observed during much of 2005. Mean incremental 2-year growth was better for all strains combined at the mid- (62.8 mm) and down-river (61.5 mm) sites than upriver (49.4 mm), probably because of occasional marginal salinities at the upriver site during year 1. Two-year growth of DEBYs (64.5 mm) was better than CROSBreeds (56.5 mm) or standards (52.5 mm) based on net shell-length increases. After 2 years, prevalence and body burdens of *Perkinsus marinus* in experimental oysters are increasing, and light mortalities are beginning to occur, all of which are probably related to increasing salinity and increasing infection levels in feral host populations in the deployment areas. Whether disease levels and mortalities of experimental oysters remain at acceptable levels will depend on future salinities; whether DEBYs or CROSBreeds outperform standards will depend on future salinities and also on functional genetic effects. Differences should be apparent by the end of the third year.

**OYSTER (CRASSOSTREA VIRGINICA) REEF RESTORATION IN THE BELLAMY RIVER, NH:
INITIATION OF A MULTISPECIES PROJECT**

Holly Abeels (Jackson Estuarine Laboratory - University of New Hampshire), Ray Grizzle (Jackson Estuarine Laboratory - University of New Hampshire), Jennifer Greene (Jackson Estuarine Laboratory - University of New Hampshire), Krystin Ward (Jackson Estuarine Laboratory - University of New Hampshire)

The eastern oyster (*Crassostrea virginica*) plays an important ecological role in the Great Bay estuarine system, where the populations have declined due to disease and other factors. In 2000, the New Hampshire Estuaries Project (NHEP) developed a management plan with a goal of increasing oyster populations by adding twenty acres of "restored" oyster bottom by the year 2010. The current project was developed in collaboration with NHEP and the City of Dover, NH to contribute to this goal and to begin multispecies habitat restoration in the Bellamy River estuary. The river bed was surveyed in fall 2004 using a towed video and DGPS system and bottom habitats classified to determine the extent of hard bottom available for planting of "seed" oysters on cultch. Ten million larvae spawned from Great Bay broodstock were set onto shell cultch in two 3,000-gallon fiberglass tanks located at Jackson Estuarine Laboratory in July 2005. Once settlement had occurred, the spat were transferred to a nursery raft located in an adjacent cove. After 3 months, approximately 400,000 spat (~40 mm shell height) had survived and were transferred to the study site where twelve mini-reefs were created in a 6000-m² area and one reef created in a 200-m² area. This area is located adjacent to an ongoing eelgrass restoration project, which will allow a multispecies community to develop. Future work will include sampling with towed video, counts and measurements of live oysters, and set out of mussel recruitment devices for transfer to multispecies habitat.

OYSTER RECRUITMENT AND EARLY GROWTH PATTERNS IN THE BARATARIA ESTUARY

Cassandra D. Addison* (Department of Biological Sciences, Nicholls State University), Earl J. Melancon (Department of Biological Sciences, Nicholls State University)

We document temporal and spatial spawning, recruitment, and early growth of oysters within the historically productive zone of the Barataria Estuary from April 2004 to November 2005. The study area falls within the outfall of the Davis Pond Mississippi River Diversion, which has flowed minimally with no measurable impact to oysters. Hence, data reflect responses to natural conditions, especially the synergism of salinity and temperature. During the study there were two reduced-salinity events (<5ppt), accompanied by two tropical storms and three hurricanes.

There was a spawn and recruitment failure throughout the estuary in May/June '04 due to low salinities (<5ppt). Oysters reabsorbed gonad tissue and died by mid-summer, except at the most down-estuary site. The May '05 spawn proceeded normally, but recruitment failed. Recruit failure may be due to a lack of food availability. Fortunately, successful fall spawn and recruitment events occurred in 2004 and 2005. Preliminary findings suggest a spatial significance in recruitment abundance, with down-estuary areas best.

Individual fall '04 spat were tagged and placed in cages at two sites. In 11 months, from October '04 to August '05, the down-estuary site produced oysters with a mean length of 62.7 ± 7.5 mm, while up-estuary oysters grew to 47.5 ± 7.9 mm. Fall-recruited spat can grow to a desired commercial-sized seed in one year, with the more down-estuary site showing faster growth.

This information becomes crucial for natural resource managers and oystermen, especially during low-salinity conditions. Adaptive up-estuary to down-estuary management strategies for seed can be developed to allow diversions and oysters to co-exist.

GROWTH AND GONAD PRODUCTION OF THE SEA URCHINS *HEMICENTROTUS PULCHERRIMUS* AND *ANTHOCIDARIS CRASSISPINA* AMONG ALGAL SERE

Yukio Agatsuma (Graduate School of Agricultural Science, Tohoku University), Hisayuki Arakawa (Tokyo University of Marine Science and Technology), Kazuya Taniguchi (Graduate School of Agricultural Science, Tohoku University)

To clarify the differences in the growth and gonad size of the sea urchins *Hemicentrotus pulcherrimus* and *Anthocidaris crassispina* among algal sere, the study was conducted in July 2002 at a fucoid bed and a kelp bed in Gobo and two algal turfs in Mihama, Wakayama Prefecture in southwestern Japan. The growth of *H. pulcherrimus* in the fucoid bed and the kelp bed, where the large perennial *Myagropsis myagroides* and *Ecklonia cava* at the climax stage dominated, respectively, are higher than that in the algal turfs dominated by the small perennial articulated corallines at the seral stage. While, the most rapid growth of *A. crassispina* was observed in the fucoid bed. The gonad indices (gonad wet weight x100/body wet weight) of both species of the sea urchins were highest in the fucoid bed. These results suggest that growth and gonad production of *H. pulcherrimus* and *A. crassispina* are affected by algal sere as reported in *H. pulcherrimus* and *Strongylocentrotus nudus* in northern Japan, although they differed among dominant species of algae at the climax stage.

ASSESSMENT OF THE ABILITY OF *CRASSOSTREA ARIAKENSIS* HEMOCYTES TO KILL *PERKINSUS MARINUS* IN VITRO

Mohammad R. Alav i (Center of Marine Biotechnology, UMBI, University of Maryland), Jose A. F. Robledo (Center of Marine Biotechnology, UMBI, University of Maryland), Eric J. Schott (Center of Marine Biotechnology, UMBI, University of Maryland), Keiko Saito (Center of Marine Biotechnology, UMBI, University of Maryland), Satoshi Tasumi (Center of Marine Biotechnology, UMBI, University of Maryland), Gerardo R. Vasta (Center of Marine Biotechnology, UMBI, University of Maryland)

The American oyster, *Crassostrea virginica*, has been reduced to such low numbers in the Chesapeake Bay that the introduction of the Asian oyster *C. ariakensis* is being considered by the industry, and state and federal agencies. To assess the susceptibility of *C. ariakensis* to *Perkinsus marinus* (Dermo) we examined the capacity of *C. ariakensis* hemocytes or plasma to kill or inhibit proliferation of *P. marinus* trophozoites, and compared it to that of *C. virginica*. *P. marinus* trophozoites were exposed in vitro to oyster hemocytes or plasma (at six infection ratios) for 24 and 72 hours and the parasite's ability to proliferate after the exposure was assessed by culture in nutrient medium for four weeks, with weekly measurements of cell densities. Although both *C. virginica* and *C. ariakensis* hemocytes apparently reduced the viability of most of the exposed parasites, even at the ratio of 16:1 (hemocytes: parasites) a small proportion of the parasite survived and proliferated upon addition of culture medium.

To rigorously discriminate adherent trophozoites from those phagocytosed by the oyster hemocytes, we developed a method based on a chloromethylbenzamido derivative of a dialkylcarbocyanine dye (CM-DiI), a lipophilic molecule that can remain highly fluorescent when incorporated into cell wall and can be quenched by trypan blue if the parasite remain extracellular. Using this method, both degraded and intact parasite could be

detected within hemocytes 24 hours post-exposure, confirming our previous results. (Supported by grants from the Maryland DNR and NOAA)

EFFECTS OF HARMFUL ALGAE ON OYSTER GROWTH

J. Alexander (University of MD Center for Environmental Science), D. Meritt (University of MD Center for Environmental Science), S. Alexander (University of MD Center for Environmental Science), D. Stoecker (University of MD Center for Environmental Science), P. Glibert (Effects of harmful algae on oyster growth)

Introduction of the Asian oyster, *Crassostrea ariakensis*, to Chesapeake Bay is of considerable interest in the broader plans for Bay restoration. Overfishing, loss of habitat, and disease have caused dramatic losses in the native oyster, *Crassostrea virginica*, and it is thought that the Asian oyster may succeed where the native oyster has not because of its high growth rates, among other factors. One of the manifestations of poor water quality in Chesapeake Bay is the development of harmful algal blooms. Critical to an understanding of the potential success of the Asian oyster in Chesapeake Bay is knowledge of the impact of common harmful algae on oyster growth. The first phase of this investigation included a comparison of the effect of harmful algae on spat growth, measured as shell growth, of both *C. virginica* and *C. ariakensis*. Effects of exposure to *Prorocentrum minimum* were compared to that of *Isochrysis galbana*, one of the standard hatchery feed organisms, and a non-fed control treatment. Valve height of cultchless test oysters, prepared by grinding away a portion of the shell periphery, following guidelines from the EPA Oyster Acute Toxicity Test, was measured before and after 96 hrs exposure to each treatment. Feces, pseudofeces, and gut contents were also collected from sample animals to compare digestion. Initial results indicate that *C. virginica* was impacted negatively by *P. minimum* to a greater extent than was *C. ariakensis*.

IN VITRO INVESTIGATIONS OF QUAHOG PARASITE UNKNOWN (QPX)

Bassem Allam (MSRC, Stony Brook University, Stony Brook NY 11794-5000), Deenie M. Buggé (MSRC, Stony Brook University, Stony Brook NY 11794-5000), Mickael Perrigault (MSRC, Stony Brook University, Stony Brook NY 11794-5000)

Quahog parasite unknown (QPX) has been successfully isolated from clams from different geographic locations in the Northeast. This study uses an *in vitro* approach to investigate the virulence and specificity of different QPX isolates. A fluorometric growth measurement technique and a neutral red cytotoxicity assay were used to investigate interactions between QPX and components from different bivalve species and strains. Our experiments demonstrate that QPX produces extracellular virulence factors that are cytotoxic to *M. mercenaria* hemocytes. This cytotoxicity may play an important role in supporting QPX infection and proliferation within the host. Experiments also indicate that QPX growth in *M. mercenaria* is tissue-specific and that some tissues possess anti-QPX properties. For example, host foot tissue supports *in vitro* QPX growth while growth in mantle or gill tissue is strongly inhibited. Bivalve plasma also contains anti-QPX factors which may play a role in host defense. Plasma from species not known to develop QPX disease contains higher anti-QPX activity than *M. mercenaria* plasma. The *in vitro* inhibitory effect of plasma seems however to be time-dependent and lasts up to two days since further incubation results in an enhancement of QPX growth. These and future *in vitro* investigations will increase our understanding of QPX infection and disease development. For instance, studies targeting tissues where anti-QPX activities are localized may provide a good strategy for the determination of biological bases of QPX resistance in clams.

RISKY BUSINESS: PROSPECTIVE LOOK AT TRIPLOID *C. ARIAKENSIS* AQUACULTURE

Standish K. Allen Jr. (Virginia Institute of Marine Science)

Among the options under consideration by the current Environmental Impact Statement (EIS) process on the introduction of *C. ariakensis* to Chesapeake Bay is that of aquaculture of triploid *C. ariakensis*. The premise for this option is that sterile triploids can eliminate or lessen the risk of inadvertent introduction. Triploidy is produced by crossing tetraploid with diploid brood stock, yielding high proportions of triploids – but not 100%. Our experience with certifying 8 batches (average sample size for each certification: 3558) of triploids produced in this way

indicates that, on average, batches are $99.86 \pm 0.094\%$ (95% CI) triploid. Essentially, the risks posed by triploid aquaculture boils down to the risk of reproduction from the 1 in 1000 diploids scattered among the crop. We have overseen two major industry aquaculture trials with triploid *C. ariakensis*. Because of Federal permitting requirements by the Army Corps of Engineers for these trials, various other Federal agencies, as well as States and NGOs, have weighed in on provisions for permitting. Risk has been quantified by a simple probability/demographic model to estimate the number of surviving spat that result from various deployment options. Moreover, these risk calculations have been deemed cumulative, such that those risks posed in a previous industry trial with triploids are summed with those for subsequent ones. Clearly, this approach leads to spiraling impossibility to continue these trials, and seemingly, excludes the opportunity for commercial scale aquaculture. A more "liberal" approach to engaging triploid aquaculture and dealing with its concomitant risks will be discussed.

SHORT-TERM EVALUATION OF MORTALITY FROM BONAMIA SP. INFECTIONS IN CRASSOSTREA ARIAKENSIS AT TWO LOCATIONS IN SOUTHEASTERN NORTH

Troy Alphin (Center for Marine Science; UNC-Wilmington; Wilmington, NC), Ne 28 Center For Marine Science; Unc-wilmington; Wilmington (Department of Biology and Marine Biology; UNC-Wilmington), Ryan B. Carnegie (Virginia Institute of Marine Science; Gloucester Point, VA), Martin H. Posey (Department of Biology and Marine Biology; UNC-Wilmington)

Crassostrea ariakensis (the Suminoe oyster) has been proposed as a potential aquaculture species for several states along the Atlantic coast of the United States, including Maryland, Virginia, and North Carolina. A 2003 trial of *C. ariakensis* in Bogue Sound, North Carolina, resulted in high mortality due to infection by *Bonamia* sp. The presence of *Bonamia* sp. may represent a significant impediment to the future culture of *C. ariakensis* along the east coast. As part of a field study evaluating the presence of *Bonamia* sp. in coastal North Carolina, *Ostreola equestris*, a species that may serve as a reservoir host, was sampled from a variety of locations from Bogue Sound to Wilmington. *Bonamia* sp. was detected in the Wilmington area in July 2005 and juvenile triploid *C. ariakensis* were deployed at two Wilmington locations in late September to determine if parasite transmission to this non-native species would occur. The first site was a research bottom lease, at the mouth of Hewletts Creek, NC and the location where *Bonamia* sp. was observed in *O. equestris*, while the second site was located 3 km south along the ICW at a site where *O. equestris* had not been noted. *C. ariakensis* were deployed for eight weeks with weekly monitoring of *Bonamia* sp. prevalence and mortality, and biweekly monitoring of growth. *Bonamia* sp. infections in *C. ariakensis* were noted at both locations, with a higher *Bonamia* sp. prevalence at the research lease site. Peak mortality approached 40% during the 5th week of the study.

IN VITRO PROTEASE PRODUCTION BY QPX

Robert S. Anderson (University of Maryland Center for Environmental Science), Tracey M. Luskey (University of Maryland Center for Environmental Science), Maureen A. Strauss (University of Maryland Center for Environmental Science)

QPX secretes virulence factors, including proteases that contribute to success in its host. In vitro production of QPX proteases was followed by zymography for 4-6 wks, using tris-glycine gels with 0.1% gelatin incorporated as a substrate. Little or no protease activity was detected in unsupplemented Kleinschuster's medium, in which QPX was cultured at 11o, 20o or 27 o C. However at 20o C, supplementation with fetal bovine serum (FBS) resulted in the production of 6-7 QPX-dependent proteases, plus 4-5 FBS-associated proteases. After 6 wks incubation, QPX proteases included a >200 kD band plus bands at ~70, 51 and 46 kD; other proteases were weakly or occasionally expressed. The 46 kD band was consistently the major QPX protease. Expression of QPX 46 kD protease showed temperature dependency: it was maximally (+++) expressed by 1 wk at 27o C, but seen as a faint (+/-) band after 3 wks at 11o and 20o C. Experiments were carried out to determine if the presence of host tissue extracts in the medium would modify the expression profile of QPX proteases. FBS and homogenate proteases interacted to produce strong bands of activity that made observation of QPX proteases impossible. Therefore, homogenate effects were measured in FBS-free medium. Unique, active (++) ~60 & 65 kD proteases were seen throughout the 6 wk course of the homogenate-QPX study. The major (46 kD) protease in FBS-supplemented cultures was weakly



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expressed, and only after 3 wks. These results suggest that the microenvironment of QPX may influence its pathogenicity.

RETURNING TO SALTWATER POND CULTURE OF CRASSOSTREA VIRGINICA

William D. Anderson (South Carolina Department of Natural Resources), William A. Cox (Island Fresh Seafood, Inc.), Jack M. Whetstone (Clemson University)

Using saltwater ponds to cultivate oysters can be traced back to the Romans in the first century B.C., and even earlier to Chinese aquaculture. In South Carolina and east coast states, growing large, single eastern oysters in saltwater ponds and abandoned impoundments has been practiced since the 1800s. SC's renowned "mill pond oyster" floated as spat on logs into saw-mill ponds, grew near the surface, dislodged, and if it survived in the mud bottom environment, matured into an epicurean delight. Oyster culturists observed pond oysters' adductor muscles to be at least one-third larger than its estuarine sibling, ostensibly to avoid smothering by siltation on the mud bottom.

Saltwater ponds generate a favorable environment for controlled autotrophic production and can sustain a higher instantaneous biomass throughout the year compared to adjacent, productive estuarine waters. Through intake filtration, circulation adjustments, salinity and water level control, ponds can be intensely managed to inhibit parasites, predators and disease while accelerating growth.

Optimum conditions for pond and estuarine aquaculture of eastern oysters have evolved recently in SC due to a successful clam mariculture infrastructure seeking diversification, strong market demand for single local oysters, hatchery technology advances and the capacity to cultivate large (>57mm) juvenile oysters in a controlled environment.

Island Fresh Seafood, a SC Corporation has spawned *C. virginica* seed that has grown 63 mm in six months, considerably faster than wildstock oysters. This presentation discusses salt water pond characteristics, production of seed oysters and SC's cooperative fisheries research initiatives to engender single oyster aquaculture by commercial shellfishermen.

AN OVERVIEW OF BIVALVE POPULATION RESTORATION EFFORTS IN FLORIDA WATERS.

William S. Arnold (Florida FWCC Fish and Wildlife Research Institute)

The coastal zone in Florida is being developed at a rapid pace, placing increasing pressure on nearshore marine resources including bivalve mollusks such as scallops, clams, and oysters. In an effort to mitigate the impacts of coastal development and to maintain viable and broadly distributed populations of these species, a variety of approaches have been applied to rebuild or reestablish populations. These approaches include transplanting adults, planting seed, releasing larval or post-larval animals, and constructing habitat. Success has varied considerably depending upon the method employed, the target species, and the interactive effects of species and habitat. Among many lessons that have been learned, one of the most essential is that the cause of collapse must be identified and either ameliorated or circumvented prior to initiating a restoration effort. Otherwise, the likelihood of success is low. Because similar development pressures are either underway or imminent throughout the United States, the lessons learned in Florida should be of interest to a broad array of coastal zone scientists and managers.

DOES DISTANCE FROM SUBTIDAL CHANNEL INFLUENCE USAGE OF NEWLY ESTABLISHED INTERTIDAL OYSTER REEFS BY TRANSIENT AND RESIDENT FAUNA

Stephen J Artabane (University of North Carolina, Wilmington), Martin H. Posey (University of North Carolina, Wilmington), Troy D. Alphin (University of North Carolina, Wilmington).

The habitat value of oyster reefs (*Crassostrea virginica*), to many finfish and decapods may vary significantly with landscape factors such as proximity of adjacent habitats, distance to source areas, and reef architecture. We examined how distance of intertidal reefs from subtidal channels, low tide refuges for transient fauna, influences reef use by both resident and transient fauna. Four transects of artificial reefs were created using clean oyster shell and placed at distances of 5m, 30m, and 55m from a subtidal channel on a tidal flat in southeastern

North Carolina. Transient finfish and crustaceans were sampled during flood tide using Breder traps and surround nets. Resident species were sampled using shell excavations and sediment cores. Preliminary data suggest preferential use of reefs over non-reef controls and use of edge habitat over interior habitat for the dominant transient species: pinfish, spot and mummichog. Conflicting patterns were observed between both sampling methods for pinfish, with Breder trap data indicating preference for reefs furthest from channel and surround net data indicating preference for reefs closest to the channel. No clear pattern of use was observed for mummichog and juvenile spot. Excavations show higher settlement of oyster spat on reefs located at 5m from the subtidal channel, but no differences among distances for recruitment of mud crabs, *Panopeus herbstii*. Measurements of predation using tethered grass shrimp, *Palaemonetes pugio*, and outplanted hard clams, *Mercenaria mercenaria*, were conducted to measure actual reef utilization but results were inconclusive.

ENSO AND CRUSTACEAN POPULATIONS OF PERÚ: ARE THE LANDINGS RECORDS RELATED TO THE SEA SURFACE TEMPERATURE (SST)?

Cynthia M. Asorey (Facultad de Ciencias Exactas y naturales-UBA/CADIC/CENSOR EU), Javier Calcagno (Facultad de Ciencias Exactas y naturales-UBA/ CENSOR EU), Gustavo A. Lovrich (Centro Austral de investigaciones CADIC, Ushuaia/CENSOR EU)

The marine ecosystem of the south eastern Pacific is made up of a particular system of superficial currents that make the coasts of Chile and Peru some of the world's most productive fishing zones. There have been few studies done about the population dynamics of commercial crustaceans and ENSO. This unconcern is probably due to the low economic value of these species, when compared to other commercial ones. In this work, mean and maximum temperatures registered at the Chicama station (07° 41' S, 79° 26' W), between 1970 and 1999 were correlated with the crustacean landing records of the Instituto del Mar del Peru. We found a positive correlation between the total crustacean landings of shrimp species (*Litopenaeus spp.*, *Farfantepenaeus californiensis* y *Xyphopeneaeus riveti*) and both the annual mean temperatures ($r=0.43$, $p<0.05$) and the maximum temperatures ($r=0.39$, $P<0.05$). We also found a negative but not significant, correlation ($r=-0.22$ for the mean SST and $r=-0.28$ for the maximum SST, $p>0.05$), for the crab species (*Cancer sp.*, *Platyxanthus sp.*). These results coincide with earlier works that report on the increase of mortality rates and migrations to deeper water of crab species. Coincidentally, during the ENSO, commercial shrimps move south from off Ecuador to off Perú, where fishers adapt their fishing arts to catch shrimp.

INFLUENCE OF TEMPERATURE ON THE METABOLISM OF MUNIDA GREGARIA (= M. SUBRUGOSA) (CRUSTACEA: ANOMURA)

M.C Avalos (CADIC (Centro Austral de Investigaciones Científicas), Ushua), O. Heilmayer (Alfred Wegener Institute for Polar and Marine Research, Brem), K. Paschke (Instituto de Acuicultura - Universidad Austral de Chile, Pue)

Munida gregaria is an epibenthic galatheid crab of 5 to 7 cm total length that has a key role in the coastal subantarctic ecosystem of South America. Although plenty of information on the species related to growth, reproduction, abundance and distribution is available not much is known about physiological adaptations to subantarctic temperatures. This preliminary study aimed to determine standard and active metabolism of *M. gregaria* acclimated at three different temperatures. Animals were caught off Puerto Montt (47°S), Chile, with an underwater vacuum at ~10 m depth. Oxygen consumption of animals (n=6 per treatment; mean carapace length=13,5 mm ± 1 mm) acclimated to 6°, 12° and 18° was measured. A closed system was used for respiration measurement. Oxygen content was determined with microoptodes connected to a single channel MICROX TX3 array. After standard metabolism (defined as oxygen consumption of unfed, unstressed animals) was measured, animals were forced to move and be active. Oxygen consumption was measured to obtain active metabolism. Results show that standard metabolism of *M. gregaria* was significantly positive affected by temperature (6°= 0,105 mgO₂.h⁻¹.g⁻¹; 12°= 0,161 mgO₂.h⁻¹.g⁻¹; 18°= 0,232 mgO₂.h⁻¹.g⁻¹). Active metabolism, however, could only be measured in few animals. In the cases the animals showed high levels of activity, a significant non-temperature dependent 2 to 3 fold increases in their oxygen consumption was measured. Our data provide preliminary insight into the physiology of this key species and help setting future experiments on this species, in order to study its thermo-tolerance in a latitudinal temperature context.

INFLUENCE OF SALINITY ON THE DISTRIBUTION AND ABUNDANCE OF LARVAE OF DOMINANT OYSTER-REEF DECAPODS IN SOUTHWEST FLORIDA

Bethany M. Bachelor (Florida Gulf Coast University, Coastal Watershed Institute), S. Gregory Tolley (Florida Gulf Coast University, Coastal Watershed Institute), Scott E. Burghart (University of South Florida, College of Marine Science)

Spatial and temporal variability in recruitment of associated organisms to oyster reefs is dependent upon a number of factors including larval supply, which in turn is influenced by freshwater inflow and salinity. Distribution and abundance of larvae of the three dominant decapod crustaceans found on oyster reefs in Estero Bay, Florida-*Petrolisthes armatus*, *Eurypanopeus depressus*, and *Rhithropanopeus harrisi*-were quantified using monthly plankton tows. Sampling included stations near passes connecting to the Gulf of Mexico, in the open waters of the bay, and in association with tidal tributaries. Larval densities of the marine stenohaline *P. armatus* were greater in bay waters and near passes. Larvae of the euryhaline *E. depressus* occurred abundantly throughout the bay and in association with tidal tributaries. Larvae of *R. harrisi*, a species known to favor reduced salinities, were most abundant near tidal tributaries. Densities of *P. armatus* and *E. depressus* peaked at the end of the dry season (May) and were depressed with the onset of seasonal rains (June). In contrast, *R. harrisi* larvae were in short supply during dry months but were abundant during the wet season. Weighted mean salinity of capture was higher for *P. armatus* (29.72) and *E. depressus* (29.88) than for *R. harrisi* (6.72), and larval density was positively correlated with salinity of capture for *P. armatus* and *E. depressus* and negatively correlated for *R. harrisi*. These results suggest the importance of considering the effects of salinity on spatial and seasonal variation in larval supply in understanding recruitment dynamics of oyster-reef associates.

LIFE HISTORY CHARACTERISTICS OF THE NON-NATIVE GREEN MUSSEL, *PERNA VIRIDIS*, IN TAMPA BAY, FLORIDA.

Shirley Baker (Dept. Fisheries and Aquatic Sciences, University of Florida), Bruce Barber (Eckerd College), Jon Fajans (Keys Marine Laboratory), Patrick Baker (Dept. Fisheries and Aquatic Sciences, University of Florida)

The green mussel, *Perna viridis*, has been intensively harvested and cultured for human consumption throughout its native range and has been transplanted outside its range numerous times for aquaculture. In August 1999, *P. viridis* was identified in Tampa Bay, Florida, the first report from North America. The overall objectives of our three-year research program were to monitor and predict the spread of *P. viridis* and major interactions with native bivalve species and phytoplankton communities. Here we examine the reproduction, recruitment, growth, and survival of *P. viridis*. Monthly samples were examined histologically for reproductive status and settlement plates were used to examine recruitment. Monthly quantitative samples were collected to estimate size distribution, cohort growth and survival. Based on both histological and recruitment data, *P. viridis* in Florida, as in Asia, has a primary spawning peak in spring and a lesser peak in autumn. Patterns were strongest in peak habitat (based on *P. viridis* density); the autumn peak timing or intensity varied in areas of lower *P. viridis* density. Growth rate was rapid – about 9 mm per month – and within the range of growth rates reported in native habitats. Based on size, some *P. viridis* may become sexually mature and attain fishery size (~ 70-80 mm) within one year. As demonstrated in this study, green mussels successfully reproduce in Florida, resulting in dense populations throughout Tampa Bay.

RESTORATION OF INTERTIDAL OYSTER REEFS AFFECTED BY INTENSE BOATING ACTIVITY IN SHALLOW FLORIDA WATERWAYS

Andrea Barber (University of Central Florida), Linda Walters (University of Central Florida), Anne Birch (The Nature Conservancy)

In recent years, intertidal reefs of the oyster *Crassostrea virginica* in central Florida's coastal areas have suffered extensive losses due to wakes from recreational boats. The creation and enforcement of "no wake" zones in the area are unlikely. Thus there is an urgent need for an alternative restoration strategy before oyster reefs decline past the point of no return. The goal of this project is to implement a scientifically-based restoration technique that

minimizes wake damage from recreational vessels on intertidal reefs in Canaveral National Seashore (CANA). To accomplish this, we will test a range of restoration measures to determine the optimal design that best increases: 1) the numbers of oysters, 2) 3-D structure of our intertidal reefs, and 3) biodiversity and abundances of sessile and motile species on reefs. These restoration measures will include all combinations of leveling piles of disarticulated shells on reefs, placing seagrass seaward of reefs, and deploying miniature, mobile oyster reefs (restoration mats) to provide substrate for oyster recruitment and survival. Our restoration mat design includes affixing 36 drilled oyster shells to 0.4 x 0.4 m² pieces of black mesh. After completing this experimental objective, the majority of our effort will be to implement our optimal design to increase reef dimensions to historical levels within the bounds of CANA. Our goal is to restore 15 - 20% of the 400 reefs (60 – 80 reefs) in the project area (approximately 40 acres) that have been damaged by wakes from recreational vessels.

PARTICULATE MATTER PRODUCTION ASSOCIATED WITH DEEP-WATER SUSPENDED PACIFIC OYSTER (CRASSOSTREA GIGAS) FARMS IN BRITISH COLUMBIA.

P.A. Barnes (Centre for Shellfish Research, Malaspina University-College), W. Friesen (Centre for Shellfish Research, Malaspina University-College), S. Switzer (Centre for Shellfish Research, Malaspina University-College)

The economic importance of bivalve culture in coastal areas of the northeastern Pacific is not reflected in the amount of information available on the environmental interactions of culture facilities. This study was designed to determine the fate of the particulate material produced by deep-water, suspended Pacific oyster farms in British Columbia. Data was collected quarterly for one year at 2 sites of contrasting current regimes. Seven stations, including 2 reference stations, were sampled at each site along a transect running through the centre of the oyster farm. Sediment trap data yielded deposition rates, including rates for TOC/TIC and TN. Chemical and biological parameters were measured on sediments at all sites. Water column data collected include plankton community composition, particle size and abundance, and a suite of environmental variables including salinity, temperature, dissolved oxygen, pH, turbidity/total suspended solids and chlorophyll. The results of preliminary analyses investigating relationships between depositional data, water column variables and benthic data for the 7 stations at each of the 2 sites are discussed, in addition to seasonal trends.

INVERTEBRATE BIODIVERSITY ASSOCIATED WITH DEEP-WATER PACIFIC OYSTER (CRASSOSTREA GIGAS) FARMS IN BRITISH COLUMBIA, CANADA.

P.A. Barnes (Centre for Shellfish Research, Malaspina University-College), S. Switzer (Centre for Shellfish Research, Malaspina University-College), B. Burd (Eco-Stat Research Ltd.)

Diverse invertebrate communities (“fouling” communities) are associated with BC’s deep-water, suspended shellfish culture systems. The three-dimensional structure of these systems increases habitat complexity and provides hard substrate for larval settlement. These diverse communities require investigation to determine their role in the environmental interactions of shellfish aquaculture, to document invasive species and to assist in documenting B.C.’s coastal marine biodiversity. This study monitored fouling communities associated with deep-water suspended oyster rafts at 2 sites of contrasting current regimes. Biodiversity data were collected in the summer, winter and spring; organisms were identified to species, or the lowest taxon possible using the current taxonomic literature. Community composition data are presented, along with results of preliminary data analysis investigating changes in community composition with depth, site and season.

HOW EFFICIENT IS THE BLUE MUSSEL (MYTILUS EDULIS L.) AT FILTERING EXCESS PARTICULATE MATERIAL AT AN INTEGRATED AQUACULTURE SITE?

Aaron T. Bennett (University of New Brunswick (Saint John)), Bruce A. Macdonald (University of New Brunswick (Saint John)), Fred H. Page (Fisheries and Oceans Canada; University of New Brunswick)

In 2001 a collaborative project began to investigate the viability of integrated aquaculture (finfish/shellfish/seaweed) in the Bay of Fundy. The project will help to quantify the economic and environmental benefits and serve as a model for developing integrated, sustainable aquaculture. The present study has two main objectives: 1)

characterizing the suspended particle field within and surrounding an integrated aquaculture site using various methods to capture the entire size range of suspended particulates and 2) determining the uptake and absorption of suspended particles on the integrated aquaculture site by the blue mussel, *Mytilus edulis*. We are using videography to capture the size distribution of undisturbed particles from a minimum of 100 μ m to the upper range of particle size limit. Particles smaller than the range of the video resolution are measured using a Coulter Multisizer which results in a size distribution pattern for individual particles measured. In order to obtain a more complete picture, a biodeposition rate is calculated from the feces and pseudofeces deposited by the mussels during the feeding trials. A net rate is then calculated for the amount of particulates completely removed from the system. We believe this research will support the assumption that mussels play a vital role in the environmental sustainability of integrated aquaculture in the Bay of Fundy.

OPTIMIZATION OF GROW-OUT CONDITIONS FOR THE LARGE SCALE CULTURE OF QUEEN CONCH, STROMBUS GIGAS

Anne Boettcher (Biology Department, University of South Alabama), Julie Davis (Caicos Conch Farm, Trade Wind Industries), Catherine Dyer (Caicos Conch Farm, Trade Wind Industries)

Queen conch has long been a staple within the Caribbean, and wild product is exported to an ever increasing global market. However, rising fishing pressure has resulted in the depletion of wild stocks increasing interest in both research and commercial based aquaculture programs. The Caicos Conch Farm, a facility involved in the commercial-scale culture of queen conch, has well established hatchery and juvenile rearing techniques and is in the process of optimizing onshore and offshore systems for the grow-out of conch. Recent studies focused on evaluation of pelleted test diets and determinations of feed conversion ratios. Increases in conch growth are seen with the inclusion of natural algal components to feed, and pellet formulations that include an alga base or algal mimetics are being developed. Feed conversion ratios similar to those seen for other aquaculture species are achieved with the current pelleted diet. In conjunction with an evaluation of onshore pond systems and offshore pen design, stocking densities and transfer protocols for conch in these systems are being evaluated. The technology developed through these studies will be implemented in a new grow-out facility on Grand Turk.

APPLICATION OF PHOTOPERIOD MANIPULATION AND NEW EXTRUDED DIETS IN SEA URCHIN AQUACULTURE

S. Anne Boettger (The University of New Hampshire), Charles W. Walker (The University of New Hampshire), Michael G. Devin (Peacock Canning Company), Stephen A. Watts (The University of Alabama at Birmingham), Mickie L. Powell (The University of Alabama at Birmingham), Addison L. Lawrence (Shrimp Mariculture Research, Texas A&M System)

For the past two decades, sea urchin fisheries have become important commercial resources with US exports to Japan exceeding \$150 million in 1995. However, sea urchin populations along the coasts of the US have been overexploited. In the largest US urchin fishery (State of Maine), landings and numbers of licensed urchin fishermen have declined between 1993 and 2001. To develop new aquaculture techniques and more effective diets we have used information on cellular changes occurring during gametogenesis. Individuals were exposed to ambient and July photoperiod, fed an extruded diet (Wenger) and were evaluated for gonad index, stereology of different gonadal cells, nutritive phagocyte and oocyte sizes and taste. Gonad indices (%) increased significantly in both treatments over five months. Amounts of nutritive phagocytes (%) increased significantly only under invariant photoperiod while sizes (mm) of nutritive phagocytes increased in both photoperiod treatments but were significantly greater at invariant photoperiod. Amounts of gonial cells (%) increased significantly under ambient photoperiod. Oocyte feret diameters (mm) increased in both treatments but were largest at ambient photoperiod. Gonad (roe) taste was not appealing to taste testers. Therefore seven diets of varying protein concentrations and the Wenger diet were fed to individuals maintained at invariant photoperiod for five months. This resulted in increased gonad indices in all diet treatments, but enhanced marketability of individuals for only three diets all of low/medium protein and high carbohydrate composition. Our results indicate that photoperiod manipulation and new diets can yield marketable sea urchin roe.

AN EXAMINATION OF THE BIOTIC AND ABIOTIC FACTORS INFLUENCING THE PARASITIC RELATIONSHIP OF A RHIZOCEPHALAN BARNACLE AND ITS HOST

Emily Boone (University of Richmond), Anne Boettcher (University of South Alabama), Tim Sherman (University of South Alabama), Jack O'Brien (University of South Alabama)

In the Gulf of Mexico and adjacent waters, the rhizocephalan barnacle *Loxothylacus texanus* parasitizes members of the genus *Callinectes*. However, factors that affect the distribution and prevalence of this parasite are not well understood. A series of studies has been conducted to determine what biological and physical factors influence this host-parasite relationship. *L. texanus* larvae are non-feeding and have approximately 3 days from metamorphosis to the cyprid stage for identification of a host, before their nutrient stores are depleted. During this time cypris larvae appear to be particularly sensitive to abiotic variations, including salinity fluctuations. The actual identification of a potential host appears to be mediated by chemical cues associated with the host, similar to those used by free-living barnacles. The female cypris larvae of *L. texanus* settle in response to carbohydrate or glycoprotein cues in the epicuticle layer of crab exoskeletons. Laboratory studies indicate, however, that successful settlement does not guarantee infection of the host. Results suggest that infection rates may be limited by factors such as host size, carapace content or immune response.

SPATIAL COMPETITION BETWEEN OYSTERS AND BARNACLES IN A FLORIDA ESTUARY

Michelle Boudreaux (University of Central Florida), Dr. Linda Walters (University of Central Florida)

Populations of *Crassostrea virginica* within Mosquito Lagoon, Florida have recently undergone significant die-offs. Before effective restoration protocols can be established, important questions about the ecology of these oysters must be answered. My research focused on interactions between oysters and spatial competitors that may affect the settlement, growth, and survival of *Crassostrea virginica*.

I collected species inventory data for one year using lift nets to determine the sessile species present. Barnacles (*Balanus amphitrite* and *B. eburneus*) were the most abundant competitors. To determine if *Balanus* spp. were always in competition with oysters, shells from historic shell middens were examined. Results show that there has been a five fold increase in barnacle abundance per oyster shell and appearance of an invasive barnacle, *Balanus amphitrite*, since midden shells. Thus, spatial competition between barnacles and oysters has increased in Mosquito Lagoon.

To determine how *Balanus* spp. affected settlement, growth, and survivorship of *C. virginica*, experiments were conducted in which densities of *Balanus amphitrite* and *Balanus eburneus* were manipulated on disarticulated oyster shells. Density treatments included: no barnacles (control), low, medium, and high coverage of barnacles. Laboratory settlement trials with oyster larvae were run with all shell treatments. Settlement was counted by microscopy. Treatments with oyster spat were deployed in the field to follow oyster growth and survivorship over 4 weeks. Settlement, growth, and survivorship of oysters were all affected by the presence of barnacles even in low densities. The specific *Balanus* spp. did not have an effect on oyster settlement, growth, or survivorship.

MYTELLA CHARRUANA ALONG THE ATLANTIC COAST OF FLORIDA: A SUCCESSFUL INVASION?

Michelle Boudreaux (University of Central Florida), Nancy Gillis (University of Central Florida), Dr. Linda Walters (University of Central Florida)

Biological invasions are recognized as one of the most serious problems confronting the integrity of native species and ecosystems around the world. Unfortunately, invaders often go unnoticed until they have spread extensively, making eradication difficult and very costly. Early detection and rapid response to invasions are vital to prevent potential evolutionary and ecological changes that damage both our ecosystems and our economy. *Mytella charruana*, a tropical mussel native to Mexico and South America, first appeared in the intake pipes of a

Jacksonville, Florida power plant in 1986. Fortunately, they never became established as the founder population was extirpated the winter of 1987, presumably due to cold temperatures. No new sightings of this species were recorded until 2004. Then, a population of *M. charruana* was discovered in Mosquito Lagoon, Florida (170 km south of Jacksonville) in August 2004. Since then the area has been surveyed monthly for mussel occurrences. Specimens were found August 2004 - February 2005 on manmade debris, driftwood and living oysters. No individuals were found again until August 2005. If ecological conditions are optimal for continued survival and establishment of *M. charruana*, this species has the potential to reproduce and out-compete native mussels and declining oyster populations of the area. Our goal is to better understand this invasive before this happens. Rapid actions are needed to prevent *M. charruana* from having the economic and ecological impacts of the zebra mussel *Dreissena polymorpha* and green mussel *Perna viridis*.

JUVENILE RECRUITMENT DYNAMICS OF THE PINTO ABALONE (HALIOTIS KAMTSCHATKANA) IN THE SAN JUAN ISLAND ARCHIPELGO, WASHINGTON

Joshua V. Bouma (University of Washington School of Aquatic & Fishery Science), C. Jackels (University of Washington School of Aquatic & Fishery Science), K.M. Straus (University of Washington School of Aquatic & Fishery Science), B. Vadopalas (University of Washington School of Aquatic & Fishery Science), C.S. Friedman (University of Washington School of Aquatic & Fishery Science), D.P. Rothaus (Washington Department of Fish and Wildlife)

Research has shown that adult pinto (northern) abalone (*Haliotis kamtschatkana*) are in serious decline, prompting designation as a Threatened species (Canada) and a Species of Concern (Washington State; U.S.). These declines may be due to recruitment failure; thus more information is needed about the life history of this species, especially larval and juvenile stages. We deployed 60 abalone recruitment modules (ARMs) previously shown to successfully attract juvenile abalone less than 50mm shell length in the wild, at two depths and three sites in the San Juan Island archipelago (SJI) with historically significant abalone populations. All ARMs were surveyed in situ for juvenile abundance three times over the course of 15 months. No juvenile abalone were observed, although the ARMs were successful in attracting adult abalone and other juvenile invertebrate and fish species. The lack of juvenile abalone in the ARMs may be due to Allee effects or, in the northern SJI, to elevated temperatures and lowered salinities observed from the Fraser River summer plume. To test the latter hypothesis, a 3x3 full factorial experiment was implemented to examine the influence of environmentally relevant temperature (11°C, 16°C, and 21°C) and salinity (14ppt, 23ppt and 32ppt) combinations on post-larval (3-7mm) pinto abalone survival. By day three of the experiment 100% mortality was observed in all 14ppt treatment groups regardless of temperature. Mortalities in each of the remaining temperature/salinity combinations over the 14 day study were not significantly different than controls, suggesting intolerance to low salinity in this species.

CRASSOSTREA ARIAKENSIS AND C. VIRGINICA RESPONSES TO ICHTHYOTOXIC KARLODINIUM VENEFICUM

E.F. Brownlee (Calvert High School), A.R. Place (Center for Marine Biotechnology, University of Maryland), H. Nonogaki (Center for Marine Biotechnology, University of Maryland), J.E. Adolf (Center for Marine Biotechnology, University of Maryland), S.G. Sellner (Morgan State Environmental Research Center), K.G. Sellner (Chesapeake Research Consortium)

The Eastern oyster *Crassostrea virginica* and the Asian oyster *C. ariakensis* are native and potentially introduced oysters, respectively, in the Chesapeake Bay and as such, will be exposed to the natural phytoplankton assemblages including harmful species throughout their life cycles. Recent work suggests that at least one of these prey items, the ichthyotoxic dinoflagellate *Karlodinium veneficum*, occurs frequently throughout the growth period for the oysters, thereby insuring frequent exposures for all life stages of the bivalve. *Karlodinium* produces linear polyketide toxins (karlotoxins) which elicit toxicity through sterol-dependent, non-specific pores in biological membranes. Spat and juvenile oysters of each species were exposed to a moderately toxin level [$18.5 \pm 6.2 \text{ ng ml}^{-1}$] of the dinoflagellate at environmentally-relevant cell densities and growth contrasted with rates observed on other phytoplankton species, including the spring bloom former *Prorocentrum minimum* and a phytoplankton mixture routinely used in oyster hatcheries. Growth for spat and juveniles of both oysters was significantly reduced when



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feeding on *Karlodinium*, relative to the other prey species. Experiments are continuing to identify the importance of food quality versus toxin content in oyster responses. These initial results suggest that the cosmopolitan *Karlodinium veneticum* inhibits growth and would potentially curtail oyster production within the tidal Bay and its tributaries throughout the oysters' growth periods and considering its global distribution, potential impact throughout temperate areas should be assessed.

FILTRATION CAPACITY AS A WAY TO SCALE RESTORATION OBJECTIVES: AN EXAMPLE FROM CHESAPEAKE BAY

Robert D. Brumbaugh (The Nature Conservancy, Global Marine Initiative), A. Thomas Leggett Jr. (Chesapeake Bay Foundation)

The decline of eastern oyster populations in Chesapeake Bay and elsewhere in the species' range is well documented, and efforts are underway to develop and test restoration approaches. In addition to restoring lost fisheries productivity, ecosystem services are increasingly cited as a reason for restoration. Policy makers in the Chesapeake Bay region have adopted a bay-wide goal for oyster restoration (10% of historic abundance by 2010), but little guidance exists for forecasting exactly how much restoration effort will be necessary to achieve this goal. We used the Lynnhaven River, a small tidal tributary in the southern Chesapeake Bay, as a model for both evaluating the efficacy of restoration approaches and developing scaling arguments for the larger Bay-wide restoration effort. Oyster restoration in the Lynnhaven began in 1998 and has entailed both reef habitat enhancement (~5.5 acres) and stock enhancement to increase recruitment rates. Oyster biomass, estimated from annual abundance surveys, was used to calculate a "filtration capacity" of restored reefs. We estimate that oysters on the restored reef area are capable of filtering a volume equivalent to the river's volume every 63.5 days. This approach can be used to set restoration goals at scales ranging from tributaries to entire estuaries.

IMPACTS OF THE COLONIAL ASCIDIAN DIDEMNUM SP. A ON MUSSELS, OYSTERS AND SCALLOPS

Stephan G. Bullard (University of Hartford, Hillyer College), Robert B. Whitlatch (University of Connecticut), Richard W. Osman (Smithsonian Environmental Research Center)

The carpet tunicate (*Didemnum* sp. A) is a colonial ascidian of unknown origin with rapidly expanding populations on the east and west coasts of North America. The carpet tunicate can grow on most natural and man-made hard substrata habitats (rocks, cobbles, docks, pilings, boat hulls). Large colonies form nearly solid mats that may smother benthic organisms. The carpet tunicate may be of particular concern for shellfish, and thus the aquaculture industry, as colonies can overgrow individual bivalves or completely cover material used to culture them. To determine the effect of the carpet tunicate on cultured juvenile shellfish, we deployed mussels (*Mytilus edulis*), oysters (*Crassostrea virginica*), and scallops (*Argopecten irradians*) in bags at 3.5 m depth with and without fragments of *Didemnum* sp. A. We assessed the survivorship, growth and condition index of shellfish after approximately one and two months of deployment. After one month, many shellfish in the treated bags had the ascidian growing on their shells; some were 100% covered. Preliminary data on survivorship, growth and condition index will be presented.

A MOLECULAR COMPARISON OF OYSTER HERPESVIRUSES FROM ASIA, EUROPE, AND NORTH AMERICA

Colleen A. Burge (University of Washington), Carolyn S. Friedman (University of Washington), Kimberly S. Reece (Virginia Institute of Marine Science), Jessica A. Moss (Virginia Institute of Marine Science), Tristan Renault (IFREMER)

Oyster herpesviruses (OshV) have been identified in multiple bivalve species globally. Some of these herpesviruses have been identified and described using molecular tools including Polymerase Chain Reaction (PCR), *in situ* hybridization (ISH), and nucleic acid sequencing. To date, PCR and sequence analysis have

identified possible variants. One variant (OsHV-1) collected in France was recently purified, sequenced, and morphologically characterized as a member of the family *Herpesviridae*. We have begun using PCR primer sets developed to amplify distinct regions of the OsHV-1 genome to identify possible sequence divergences in OsHV in oysters collected from two sites in China (*Crassostrea ariakensis*), one site in Japan (*C. gigas*), and one site in the United States (*C. gigas*) as compared to OsHV-1. Preliminary PCR results indicate that the OsHV identified in China and Japan is similar, but differs from OsHV-1, while samples collected in the United States are more comparable to OsHV-1. We plan to expand this analysis to include samples from sites in China and Korea where OsHV has been identified. We also plan to test additional primer sets and to sequence additional regions to better describe OsHV variants.

EASTERN OYSTER SETTLEMENT AND EARLY SURVIVAL ON ALTERNATIVE SUBSTRATES ALONG INTERTIDAL MARSH, RIP RAP, AND MANMADE OYSTER REEF

Russell Burke (Virginia Institute Marine Science: College of William & Mary), Romuald Lipcius (Virginia Institute Marine Science: College of William & Mary), Mark Luckenbach (Virginia Institute Marine Science: College of William & Mary), P.G. Ross (Virginia Institute Marine Science: College of William & Mary), Justine Woodward (Virginia Institute Marine Science: College of William & Mary), David Schulte (Army Corps of Engineers – Norfolk, VA District)

Restoration efforts with native Eastern oyster, *Crassostrea virginica*, in Chesapeake Bay have been extensive and have been impeded by substrate and recruitment limitations along with many other detrimental factors. In Lynnhaven Bay, a southern subestuary of Chesapeake Bay, the Army Corps of Engineers has partnered with the Virginia Institute of Marine Science, National Oceanic and Atmospheric Administration, and Chesapeake Bay Foundation to implement a comprehensive oyster reef restoration strategy. Surveys within the Lynnhaven Bay system indicate that artificial oyster shell reefs created in the early 1990s are producing poor to marginal oyster densities relative to densities on nearby granite and concrete riprap, and on oyster clusters along marsh-fringed shores. In this field experiment, twelve treatments simulating inter-tidal oyster habitat were placed at three sites within a tidal creek: adjacent to marsh, rip rap and a manmade oyster reef. Treatments consisted of caged and uncaged trays (0.5 m length x 0.5 m width x 0.25 m depth) of large granite, small granite, large limestone marl, small limestone marl, very small concrete/granite, and oyster shells. Granite of both sizes had the highest initial oyster recruitment across all sites. Overall, replicates showed a distinct recruitment/early survival pattern between sites: marsh > rip rap > artificial oyster reef. We therefore propose that granite may be a favorable oyster reef construction material, since it appears to enhance oyster settlement and early post-settlement survival. Additional biological benefits may accrue from granite reefs as community structure develops on the reefs.

CHANNELED-TYPE APPLESNAILS: CURRENT DISTRIBUTION, DENSITIES AND POTENTIAL THREAT TO NATURAL ECOSYSTEMS AND AGRICULTURE

Lyubov E. Burlakova (Stephen F. Austin State University, Nacogdoches, TX), Alexander Y. Karataye v (Stephen F. Austin State University, Nacogdoches, TX), David N. Hollas (Stephen F. Austin State University, Nacogdoches, TX), Leah D. Cartwright (Stephen F. Austin State University, Nacogdoches, TX)

The aquatic invasive gastropod *Pomacea canaliculata* (channeled applesnail), originally from South America, has become a major pest of rice crops throughout the Indo-Pacific Region. These large herbivorous snails can reach maturity in about two months during the summer, reproduce several times a month, and aestivate for several months, burying into the soil if the habitat dries out. By mid 2005, living populations of channeled-type applesnails were reported from Florida, Texas, California, Alabama, Arizona, and Georgia. According to the results of genetic analyses, specimens from Texas and Florida belong to the *P. canaliculata*-group. The first reproductive applesnail population in Texas was reported in 1989, and by the summer of 2005 snails were found in six southeastern counties (Harris, Chambers, Brazoria, Galveston, Fort Bend, and Waller). Reproduction of channeled-type applesnails in Texas continues for at least 8 months, from March to the beginning of November. Densities of snails in Texas vary depending on waterbody type and possibly presence of predators. The rice growing technology used in Texas



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combined with current low densities of snails are the likely reasons why agricultural damage due to applesnails has not yet been detected.

DIVERSITY AND DISTRIBUTION OF UNIONID BIVALVES IN TEXAS

Lyubov E. Burlakova (Stephen F. Austin State University, Nacogdoches, TX), Alexander Y. Karatayev (Stephen F. Austin State University, Nacogdoches, TX), Robert G. Howells (Texas Parks and Wildlife Department, Ingram, TX), Daniel L. Bennett (Stephen F. Austin State University, Nacogdoches, TX)

The family Unionidae is one of the most rapidly vanishing faunal groups in North America, and habitat destruction is considered to be the most important cause for the decline. To evaluate the current status of unionid diversity in Texas we re-examined species composition and abundance of unionid in 30 waterbodies previously surveyed by the Texas Parks and Wildlife Department. The highest diversity was found in Sabine River (18 species), B.A. Steinhagen Reservoir (16), Neches River below Town Bluff Dam (14), Village Creek (14), and Sanders Creek (14). The lower San Marcos River and Guadalupe River (Central Texas) were found to support rare, endemic *Quadrula aurea*. Living rare endemic *Lampsilis bracteata* were found in Live Oak Creek, the Guadalupe River, and the San Saba River (Central Texas). Rare species of *Fusconaia* (*F. askewi* and endemic to Texas *F. lananensis*) are still present in Attoyac Bayou and Sandy Creek, and in Village Creek in Big Thicket National Preserve (East Texas). In addition, this latter creek continues to support rare *Pleurobema riddelli*. Several large reservoirs surveyed had drought- and drawdown-related low waters in recent years that presumably caused reductions in mussel abundance and diversity.

OYSTER SEX WARS: EVIDENCE FOR A 'GAMETE SINK' IF CRASSOSTREA VIRGINICA AND CRASSOSTREA ARIAKENSIS SPAWN SYNCHRONOUSLY.

David Bushek (Haskin Shellfish Research Laboratory, Rutgers University), Andrea Kornbluh (Haskin Shellfish Research Laboratory, Rutgers University), Greg Debrosse (Haskin Shellfish Research Laboratory, Rutgers University), Haiyan Wang (Haskin Shellfish Research Laboratory, Rutgers University), Ximing Guo (Haskin Shellfish Research Laboratory, Rutgers University)

Available data indicate spawning seasons for the Asian oyster *Crassostrea ariakensis* and the eastern oyster *C. virginica* overlap. Hybrids can form, but the larvae are not viable. If *C. ariakensis* are introduced into Chesapeake Bay and synchronous spawning occurs with *C. virginica*, hybridization could reduce the production of viable larvae (= gamete sink). We examined the effects of gamete age, sperm concentration, and ratios of heterospecific gametes on fertilization rate and hybridization of the two species. Hybrid fertilization rates were consistently lower than pure crosses. Fertilization rate decayed with gamete age, but occurred in gametes up to 8 hrs old. Fertilization rate also decayed with decreasing sperm density in both pure and hybrid crosses. Finally, fertilization rate declined by as much as 60% when sperm were (1) given a choice of eggs from each species to fertilize or (2) required to compete to fertilize eggs from a single species. Hence, a gamete sink will likely occur if these two species spawn synchronously. The magnitude of the gamete sink will depend in part on proximity of the two species, on gamete concentrations in the water column, and on the proportion of hybrids that form. Current efforts are enumerating the proportion of hybridization that occurred during these experiments. Molecular genetic methods to amplify ITS regions of the rRNA gene have been validated and yield two bands in pure crosses and four bands in hybrid crosses. Individual larvae are being typed to determine the proportion of hybrids formed under the various gamete mixtures.

ESTIMATION OF RECREATIONAL DUNGENESS CRAB HARVEST IN PUGET SOUND, WASHINGTON USING A TELEPHONE SURVEY OF HARVESTERS.

Jennifer Cahalan (Washington Department of Fish and Wildlife)

The recreational Dungeness crab harvest in Puget Sound exceeds 1.5 million pounds of crab annually. In order to meet fisheries management decision-making needs, estimates of recreational Dungeness crab harvest need to be precise and accurate, provided for relatively small geographic and temporal scales, and available during the recreational season. The recreational Dungeness crab fishery is characterized by relatively low effort dispersed over a large geographic area. As a result field-based methods of estimating recreational catch were inefficient and expensive. Alternate methods of estimating catch were developed and implemented in 2002.

To fish for crab in Washington State, crab harvesters are required to obtain a catch record card (CRC) from the WDFW. A telephone survey of CRC-holders is used to collect the data used in catch estimation. Catch estimates are generated approximately 3 to 4 weeks after the survey begins for each of Puget Sound's 11 catch areas. A minimum of two surveys are conducted each season. This method provides timely in-season estimates of harvest at relatively low cost.

In this presentation the details of the methods will be discussed, including treatment of missing data, precision of estimates, cost, and potential improvements.

INBREEDING EFFECTS ON GROWTH AND SURVIVAL IN A NATURALIZED POPULATION OF THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*) REVEALED USING M

Mark D. Camara (USDA- Agricultural Research Service, Newport, Oregon USA), Sanford Evans (Molluscan Broodstock Program, Newport, Oregon USA), Chris Langdon (Molluscan Broodstock Program, Newport, Oregon USA)

Inbreeding is an important factor influencing mating systems, dispersal mechanisms, and variation in life history traits within and among populations and thus has profound implications in evolutionary biology, selective breeding, conservation biology, and even medicine. In theory, high fecundity species such as elm trees and oysters are expected to have higher genetic load and consequently more severe inbreeding depression than low fecundity species, and previous studies have confirmed that self-fertilization, brother/sister matings, and cousin/cousin matings in *C. gigas* produce progeny with lower growth and survival than non-consanguineous pairings of parents. It is unclear, however, whether these effects can be extrapolated to the lower levels of consanguinity expected in natural populations with random mating. We studied inbreeding in a naturalized population of Pacific oysters using three molecular marker-based estimates of parental relatedness calculated from multi-locus microsatellite genotypes. Using analysis of covariance approaches, we found significant relationships between estimators of parental genetic similarity and components of fitness. As expected, more highly related parents produced progeny with lower survival (i.e. inbreeding depression). Surprisingly, however, the surviving progeny of genetically similar parents had higher growth (outbreeding depression or heterosis) than less related parental pairs. We discuss potential mechanism underlying these patterns and their implications for aquaculture and selective breeding.

HAEMOLYMPH PROTEOMIC APPROACH TO ANALYSE DIFFERENCES IN SUSCEPTIBILITY TO BONAMIOSIS BETWEEN OYSTER STOCKS

Asunción Cao (Centro de Investigaciones Mariñas, Xunta de Galicia, Spain), José Fuentes (Centro de Investigaciones Mariñas, Xunta de Galicia, Spain), Antonio Villalba (Centro de Investigaciones Mariñas, Xunta de Galicia, Spain)

The parasitic protozoan *Bonamia ostreae* causes oyster *Ostrea edulis* mass mortalities throughout the European Atlantic coast. The parasite is phagocytosed by oyster haemocytes but they are not able to kill it; the parasite divides in haemocyte cytoplasm causing the rupture of the host cell. Differences in susceptibility to bonamiosis between *O. edulis* stocks has been proved. Furthermore, the oyster *Crassostrea gigas* is resistant to bonamiosis. Separation of the proteins of oyster haemolymph by two dimensional electrophoresis was tuned up resulting in multiple proteic spots on polyacrilamide gels. This proteomic approach is being applied to analyse differences in susceptibility to bonamiosis between *O. edulis* stocks and between *O. edulis* and *C. gigas*. The *O. edulis* stocks used in the comparisons are: Rossmore (strain selected for resistance against bonamiosis), oysters from Tralee Bay (Ireland) where the parasite had not previously been detected, and oysters from Ría de Ortigueira (Galician, NW Spain) where *B. ostreae* is present since early 1980s. Comparison between infected and uninfected oysters is also included. Application of specific commercial software makes easy qualitative and quantitative comparison between gels

corresponding to haemolymph of each oyster group. Early results have shown spots exclusive of each group and other spots that are common to every oyster group. Identification of the proteins in the spots could contribute to explain differences in susceptibility and even provide the key for resistance to bonamiosis.

POTENTIAL IMPACT OF BONAMIA SP. ON CRASSOSTREA ARIAKENSIS IN CHESAPEAKE BAY AND NORTH CAROLINA.

Ryan B. Carnegie (Virginia Institute of Marine Science), Nancy A. Stokes (Virginia Institute of Marine Science), Corinne Audemard (Virginia Institute of Marine Science), Eugene M. Bureson (Virginia Institute of Marine Science), Melanie J. Bishop (University of North Carolina Institute of Marine Sciences), Charles H. Peterson (University of North Carolina Institute of Marine Sciences), Ami E. Wilbur (UNC Wilmington Center for Marine Sciences), Troy D. Alphin (UNC Wilmington Center for Marine Sciences), Martin H. Posey (UNC Wilmington Dept. of Biology and Marine Biology)

A *Bonamia* sp. emerged in 2003 as the cause of mortality (> 85%) among experimental seed *C. ariakensis* in Bogue Sound, North Carolina. With introduction of this oyster to Chesapeake Bay proposed, this epizootic gave urgency to investigations into the nature of bonamiasis in the mid-Atlantic: its annual timing and range, environmental limitations, and the identity and distribution of parasite reservoirs. Field studies included *Bonamia* sp. monitoring in *C. ariakensis* deployments along a salinity gradient from Bogue Sound, and at distant coastal sites; in serial deployments of seed *C. ariakensis* to upwellers on Bogue Sound; and among *Ostreola equestris* from Bogue Sound and Wilmington, NC. Laboratory trials have explored effects of reduced salinity on existing *Bonamia* sp. infections. Epizootics recurred in 2004-2005, generating infections for laboratory use and allowing an initial portrait of *Bonamia* sp. to be developed. This parasite causes acute disease and mortality in smaller/younger *C. ariakensis* (< 40 mm) in higher salinity coastal waters during warmer months. Infections may regress in winter. It is known to occur only at Bogue Sound and Wilmington, > 100 km to the southwest. It occurs in *O. equestris* in both places, but this oyster may not be a functional reservoir for *Bonamia* sp., or its only other host. In the laboratory, infections were purged at 20 and 10 psu, further suggesting that lower estuarine salinities (such as in Pamlico Sound, where *Bonamia* sp. has been notably absent from a mesohaline *C. ariakensis* nursery site, or Chesapeake Bay) may limit *Bonamia* activity.

DEVELOPING STRATEGIES TO REDUCE THE IMPACT OF THE BORING SPONGE CLIONA CELATA ON CULTURED EASTERN OYSTERS CRASSOSTREA VIRGINICA.

Claire Carver (Mallet Research Services), André Mallet (Mallet Research Services)

It has long been recognized that the boring sponge (*Cliona celata*) can cause significant economic losses to those involved in the harvesting of wild oyster populations. It is not clear, however, whether the presence of a boring sponge population may act as a source of infection to cultured oysters grown in floating bags or on oyster tables.

Preliminary surveys conducted in one bay in northern New Brunswick, Canada, suggested that in some areas 35% of the off-bottom cultured oysters showed evidence of shell degradation associated with boring sponge infection.

The development of strategies to mitigate the impact of this organism is hampered by the lack of basic information on growth rates, spawning activity, and modes of infection. We report on the preliminary results from an ongoing multi-year project to document the life-history characteristics of this biofouling species and evaluate the effectiveness of various treatment strategies.

PREVALENCE AND GEOGRAPHIC DISTRIBUTION OF A DUNGENESS CRAB CANCER MAGISTER MICROSPORIDIAN PARASITE

Richard K. Childers (Washington Department of Fish and Wildlife), Paul W. Reno (Oregon State University, Hatfield Marine Science Center), Robert E. Olson (Oregon State University, Hatfield Marine Science Center)

The prevalence and geographic distribution of *Nadelspora canceri* a microsporidian parasite of the Dungeness crab *Cancer magister*, was studied and prevalences were analyzed by sex and size of host and date of collection. *Nadelspora canceri* was widely distributed along the United States Pacific Coast in estuaries from Bodega Bay,



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California to southern Washington. Prevalence of the parasite was high in crabs from most estuaries sampled between 1991 and 1994, ranging from a low of 0.4% (Gray's Harbor, Washington) to 41.2% (Tillamook Bay, Oregon) with an overall mean of 14%. Crab examined from Grays Harbor between 2002 and 2004 indicate the prevalence rate had increased from 0.4% to greater than 14%. The parasite also occurred offshore of California and Oregon, but was not found in Puget Sound, Washington or in Glacier Bay, Alaska. The prevalence of infection in crabs captured in the open ocean was low (0.3%). In crabs collected nearshore at estuary mouths prevalence was intermediate between estuarine and open-ocean levels. The infection was most prevalent in 2 year old crabs (13 cm carapace width), and males had 2.5 times the rate of infection than did females.

The mortality of laboratory-held crabs naturally infected with *N. canceri* was compared to that of uninfected crabs and significantly higher mortality was observed for infected crabs. *Nadelpora canceri* infections were established in juvenile and adult crabs that were fed parasite spores in laboratory experiments indicating that transmission is direct and intermediate hosts or vectors are not required for transmission.

HABITAT ATTRIBUTES ASSOCIATED WITH BAY SCALLOPS IN AN AREA WITH ACTIVE RESTORATION

Marnita M. Chintala (U.S. EPA, ORD/NHEERL, Atlantic Ecology Division), Eric J. Weissberger (U.S. EPA, ORD/NHEERL, Atlantic Ecology Division), David W. Grunden (Town of Oak Bluffs, MA), Elizabeth Hinchey (Illinois-Indiana Sea Grant, Purdue University), William G. Nelson (U.S. EPA, ORD/NHEERL, Atlantic Ecology Division), Russell Ahlgren (U.S. EPA, ORD/NHEERL, Atlantic Ecology Division), Michael Charpentier (CSC Corporation, Narragansett, RI)

Habitat quality and quantity are important factors to maintain bay scallop (*Argopecten irradians*) populations; however, data linking habitat attributes to bay scallop populations are lacking. This information is essential to relate habitat alteration to the decline of bay scallop populations and to guide restoration efforts to reverse declines. Initial results of 30 dive surveys conducted in September 2005 in Lagoon Pond, Martha's Vineyard, MA, (538 acres) indicate a correlation between scallop abundance and total plant cover (macroalgae and eelgrass combined), depth, and sediment type. Scallop abundance was highest (15.42 scallops m⁻²) at a site where average total plant cover was 24%. Most scallops were found in shallower areas (depth 1-5 m) along the edge of Lagoon Pond, with deeper, unvegetated, sites devoid of scallops. Shell height (measured from umbo to opposite edge) ranged from 10 to 90 mm, with larger scallops present in the northern and eastern pond areas. Height distribution was primarily unimodal; however, a few locations had bimodal distributions. Scallop abundance will be related to restoration methods (e.g., seeding, spat bags) in the pond to assess how these activities have influenced the relationship between bay scallops and habitat.

EFFECTS OF TRICLOSAN ON THE OYSTER PARASITE, PERKINSUS MARINUS AND ITS HOST, THE EASTERN OYSTER, CRASSOSTREA VIRGINICA: A COMPAR

Fu-lin E. Chu (Virginia Institute of Marine Science), Eric D. Lund (Virginia Institute of Marine Science), Jennifer Littell (Virginia Institute of Marine Science)

While causing minimal effect on oyster hemocyte viability, our previous study showed that the antibiotic, triclosan, not only inhibits growth and greatly reduces cell viability of in vitro cultured *Perkinsus marinus*, but also inhibits the parasite's fatty acid synthetic ability. Due to the great importance of temperature on disease progression in the field, we tested the effects triclosan on the viability of *P. marinus* meronts, the primary disease transmission stage and oyster hemocytes at high and low temperature. Parasite or oyster hemocytes were exposed to triclosan (0, 2, 5 or 10 mm) for 24 hr and then their viabilities were assessed by MTS/PMS assay. Exposure of *P. marinus* meronts to 2, 5, or 10 mm triclosan at 20, 26 and 28°C significantly reduced their viability (40–60%). At 13°C, *P. meronts* had a better triclosan tolerance, with viabilities reduced to 67-73.4% after 24 hr exposure. Oyster hemocytes treated with triclosan exhibited mortalities of less than 10% at triclosan concentrations of £10 mM at 13°C. No significant

($p < 0.05$) reduction in viability relative to the controls occurred at triclosan concentrations from 2 to 10 mM at 26°C. However, exposure of hemocyte to triclosan at 28 °C, viability reduced to less than 63% at a concentration of 10 mM. Triclosan exposure did not appear to affect the reactive oxidative intermediate production in zymosan-stimulated and unstimulated hemocytes.

SC'S COMPREHENSIVE INTERTIDAL OYSTER ASSESSMENT USING HIGH RESOLUTION IMAGERY FOR MANAGEMENT AND RESTORATION

L.D. Coen (MRRI, SCDNR), K.E. Schulte (MRRI, SCDNR), G.M. Yianopoulos (OFM, SCDNR), R.F. Van Dolah (MRRI, SCDNR), W.D. Anderson (OFM, SCDNR), M.A. Finkbeiner (NOAA Coastal Services Center), W.R. Stevenson (NOAA Coastal Services Center)

SCDNR is currently undertaking a state-wide assessment of its oyster resources as part of a multi-year, collaborative effort with NOAA's Coastal Services Center (CSC) and the U.S. Geological Survey. Using a previously developed approach for analyzing multispectral ¼ m digital imagery, we are processing the finalized imagery (digital orthophoto quarter quads) using Feature Analyst[®] and ERDAS's Imagine[®] to derive oyster reef location (presence-absence), areal extent and proportion of vertical shell coverage within a bed's boundaries. Two teams randomly survey portions of 60 DOQQs using shallow draft boats at or near MLW for verification of the processed imagery. Field efforts also utilize Trimble Pathfinder Pro XRTM surveying units to measure reef length and position, as well as to note oyster density ('strata') and average reef width. Real-time Digital8⁰ video is recorded for each reef and is post-processed to determine estimates of percent vertical oyster coverage in defined time segments. Helicopter flights are also used for inaccessible areas. Post-processed imagery is validated by identifying beds that are correctly and incorrectly identified at a predefined accuracy level. This project will allow us to: (1) complete future evaluations by periodically re-flying and reassessing the state's shellfish beds; (2) provide both SCDNR and other interested users with detailed imagery available for a manifold of other uses; and (3) focus our oyster bed restoration efforts relative to current state management plans and status and trends analyses.

APPROACHES TO SMALL-SCALE OYSTER RESTORATION: SITE CRITERIA AS A MEANS OF DETERMINING OPTIMAL METHODS FOR PUBLIC INVOLVEMENT

Loren D. Coen (Marine Resources Research Institute, SCDNR), Rob Brumbaugh (The Nature Conservancy, Global Marine Initiative), Nancy H. Hadley (Marine Resources Research Institute, SCDNR)

Crassostrea virginica reefs were once a dominant feature of most Atlantic and Gulf coast estuaries, but have drastically declined in many areas across the U.S. Once valued primarily as a fishery resource, oysters are now recognized as key elements of many estuarine ecosystems, with a diverse set of attendant ecosystem services. Large- and small-scale restoration of subtidal and intertidal oyster habitats is ongoing in most Atlantic and Gulf coast states. For small-scale community-based restoration projects, an array of restoration approaches are in use, each of which has relative merits and limitations. Examples of these approaches include oyster gardening for stock enhancement, deployment of prefilled oyster shell bags for intertidal restoration, and community-based shell recycling. We discuss the characteristics of these approaches, as well as several other deployment methods under consideration, and examine the logistical benefits and limitations of each. We also identify specific site criteria such as recruitment or substrate limitation that may be useful for identifying the most appropriate methodology for practitioners to employ in small-scale restoration projects.

MOLECULAR GENETIC VARIATION AMONG QPX ISOLATES

Jackie L. Collier (Marine Sciences Research Center, Stony Brook University), Hua Qian (Marine Sciences Research Center, Stony Brook University), Qianqian Liu (Marine Sciences Research Center, Stony Brook University), Bassem Allam (Marine Sciences Research Center, Stony Brook University)

QPX has caused significant mortality of hard clams, *Mercenaria mercenaria*, in many locations along the east coast of North America since the 1960's, and a new outbreak of QPX occurred in Raritan Bay NY during the summer of 2002. We have cultured new isolates of QPX from Raritan Bay clams collected in 2003 and 2004 and are using them, along with the Massachusetts isolate ATCC50749 and new Massachusetts QPX cultures isolated in 2005, to investigate the molecular genetic variability within QPX. We have found no sequence variability in the 18S and 5.8S rRNA genes from 6 New York isolates and ATCC50749. In contrast, the rRNA operon intergenic transcribed spacers, ITS1 and ITS2, showed a great deal of variability. Interestingly, the variation among ITS1 and ITS2 sequences from a single isolate was as great as the variability among the eight QPX isolates examined (7 from NY and ATCC50749). This result shows that the many copies of the rRNA operon in the QPX genome are not homogenized by gene conversion quickly enough to overcome the accumulation of sequence variation, and may suggest that QPX reproduces predominantly asexually. Sequences of 4 mitochondrial protein-coding genes also showed no sequence variability within or between the four isolates (two from NY and two from MA) examined. We will also present data from our current efforts to sequence non-coding regions of the mitochondrial genome.

PERKINSUS MARINUS INFECTION IN OYSTERS FROM SOUTHEASTERN NORTH CAROLINA TIDAL CREEKS WITH VARYING WATER QUALITY

Sara L. Colosimo (University of North Carolina Wilmington), Martin H. Posey (University of North Carolina Wilmington), Troy D. Alphin (University of North Carolina Wilmington)

On the East coast of the United States, oysters represent a critical fishery and impact many ecosystem functions, affecting water quality and providing habitat for a variety of fish and invertebrates. However, the Eastern oyster, *Crassostrea virginica*, has experienced major mortality throughout its range; attributed in part to disease caused by the protozoan parasite *Perkinsus marinus* (Dermo). This mortality has not been as severe for intertidal oyster populations in southeastern North Carolina as it has been in other areas. Anthropogenic stressors associated with storm water runoff, such as non-point source nutrient inputs and suspended particulates, may contribute to declining water quality and affect infection rates. Exposure to such environmental stressors can adversely affect oysters and increase their vulnerability to infection. This study compares *P. marinus* infection levels in oysters from three southeastern North Carolina tidal creeks that vary in water quality characteristics. Prevalence and intensity of *Perkinsus* infection are being compared among creeks for both natural oyster populations and hatchery stock outplants using RFTM tissue assay. Data on growth, condition index, and mortality are also being compared. We predict that oysters in creeks with higher levels of nutrient inputs and particulates will have higher prevalence and intensity of infection. Preliminary results indicate that infection levels for all three creeks appear to be similar. For each creek, average infection intensity is light and infection is nearly 100% prevalent. Despite low overall infection intensities, hatchery oysters in each creek have an average mortality of 20%.

CHARACTERIZATION OF BAC LIBRARIES FROM THE OYSTERS CRASSOSTREA VIRGINICA AND C. GIGAS.

Charles Cunningham (Hollings Marine Laboratory, Charleston, SC29412.), Junichi Hikima (Hollings Marine Laboratory, Charleston, SC29412.), Robert Chapman (Hollings Marine Laboratory, Charleston, SC29412.), Matthew J Jenny (Hollings Marine Laboratory, Charleston, SC29412.), Jeff Tomkins (Clemson University Genomics Institute, Clemson, SC 29634.), Greg W Warr (Hollings Marine Laboratory, Charleston, SC29412.)

BAC libraries of two commercially and economically important species, *C. virginica* and *C. gigas*, have been developed as part of an international effort (co-ordinated by the Oyster Genome Consortium) to develop tools and reagents that will advance our ability to conduct genetic and genomic research on oysters. A total of 73,728 *C. gigas* clones with an average insert size of 150 kb were picked and arrayed representing approximately 12-fold genome coverage. A total of 55,296 clones with an average insert size of 134 kb were picked and arrayed for *C. virginica* representing approximately 10-fold coverage. The *C. gigas* and *C. virginica* libraries were screened with probes

derived from 13 and 14 randomly selected genes respectively. All of the probes tested detected multiple clones ranging from 8 - 25 clones for *C. virginica* and 5 - 50 for *C. gigas*. These numbers suggest that there is no gross bias in either library and that some of the genes probed are members of multi-gene families. We have conducted a preliminary analysis of polymorphism represented in the two libraries using gene-specific primers for PCR amplification and nucleotide sequencing from selected BAC clones. Results suggest that the degree of sequence difference between alleles is highly variable (in a gene-specific manner) and that variability is, as would be predicted, concentrated in intronic regions.

DESIGN AND CHARACTERIZATION OF A MULTI-SPECIES OYSTER CDNA MICROARRAY

Charlie Cunningham (University of New Mexico), Matt Jenny (Woods Hole Oceanographic Institute), Robert Chapman (South Carolina Department of Natural Resources), Gregory Warr (Medical University of South Carolina), Jonas Almeida (Medical University of South Carolina), Ann Chen (Medical University of South Carolina), Dave Mckillen (Medical University of South Carolina), Hal Trent (Medical University of South Carolina)

The American oyster, *Crassostrea virginica*, and the Pacific oyster, *C. gigas*, are important economic resources from both the harvesting of natural populations and aquaculture industry. In addition, because of their vital role in estuarine ecosystems oysters are a valuable model organism for environmental research. In an effort to increase our understanding of the effects of environmental pressures and disease on oyster physiology and health, an international collaboration (the Oyster Microarray Consortium) was initiated to construct a multi-species cDNA microarray consisting of expressed sequence tags from *C. virginica*, *C. gigas*, and the oyster pathogen *Perkinsus marinus*. The microarray consists of 4,460 clones from *C. virginica*, 2,320 clones from *C. gigas*, and 384 clones from the oyster pathogen, *Perkinsus marinus*. Functional annotation of the oyster clones was performed by comparison to the Gene Ontology database. As part of the initial characterization of this array, tissue specific signatures were identified by gene expression profiling both gill and hepatopancreas tissues from both species of oyster. Hepatopancreas gene expression profiles were also obtained from *C. ariakensis*, an oyster species not represented by clones on the array. The utility of the microarray for comparative studies between multiple oyster species was determined by the analysis of several hundred clones demonstrating cross-hybridization potential. Correlations between hybridization intensity and sequence homology of several hundred clones were used to estimate the level of sequence identity necessary for sufficient cross-hybridization. Results demonstrate the benefits in gene expression profiling by having multiple genes from both oyster species present on the array.

EXPRESSION ANALYSIS OF CANDIDATE LOCI FOR GROWTH HETEROSIS IN THE PACIFIC OYSTER

Jason Curole (University of Southern California)

The Pacific oyster has emerged as a model system to understand the genetic and physiological mechanisms of growth heterosis. A set of candidate loci for growth heterosis has been identified through comparative transcriptomic analysis of hybrid and inbred 6-day old oyster larvae. Evaluation of candidate loci requires discovery of polymorphisms, genetic mapping, and detailed analysis of expression patterns. *Crassostrea gigas* has a high density of single nucleotide polymorphisms and a surprisingly high density of indels (Curole & Hedgecock 2005. http://www.intl-pag.org/13/abstracts/PAG13_W026.html). Using size fragment analysis to detect indel differences between parental alleles, I have genotyped eight of these candidates in a phenotyped F₂ population. In addition, I have examined expression at several candidate loci in an F₁ population and identified SNPs in this population for analysis of allele-specific expression.

BIOENERGETICS OF JUVENILE PINK ABALONE FED FORMULATED DIETS CONTAINING DIFFERENT LEVELS OF PROTEIN AND STARCH:LIPID RATIOS

Louis R. D'Abramo (Dept. of Wildlife and Fisheries, Mississippi State University), Jessica Montano-vargas (Universidad Autonoma de Baja California), Armando Shimada (Universidad Nacional Autonoma de Mexico),

Carlos-vasquez-pelaez (Universidad Nacional Autonoma de Mexico), Maria Teresa Viana (Universidad Autonoma de Baja California)

Juvenile pink abalone *Haliotis corrugata* (0.15 g) were grown for 131 days under laboratory conditions in aerated flow through seawater systems and fed semi-purified diets containing three different levels of protein, each level containing two ranges of starch lipid ratios (1.5 to 1.87 and 3.2 to 3.6), and similar gross energy levels. Growth responses suggested a dietary protein requirement of approximately 35 % with carbohydrate being the principal source of energy (high starch to lipid ratios). Optimum dietary lipid levels appear to be principally based on the satisfaction of specific nutrient requirements. Ammonia excretion ($\mu\text{g NH}_4^+ \text{h}^{-1} \text{g}^{-1}$) ranged from 7.9 to 4.8 but was not significantly different among dietary treatments, except for the treatment containing 32 % crude protein and a low carbohydrate:lipid ratio. Energy lost due to mucus production did not significantly differ among dietary treatments. Specific dynamic action comprised nearly 50 % of the measured oxygen consumption ($\mu\text{L O}_2 \text{h}^{-1} \text{g}^{-1}$) and did not differ significantly among dietary treatments. Respiration increased during the evening, suggesting a circadian pattern that is typical of the *Haliotis* genera. Approximately 70 % of the ingested energy was lost to feces, and 25 % metabolized, with 7 to 10 % channeled to growth. A generalized pattern of utilization of dietary energy based upon collective investigations with *Haliotis* sp. is presented and suggests that 40-50 % is generally lost to fecal production and approximately 50 % of assimilated energy is lost to respiration.

THE RELATIONSHIP BETWEEN PRODUCT QUALITY AND BIOCHEMICAL COMPOSITION IN SEA URCHIN GONADS, WITH EMPHASIS ON TASTE AND TEXTURE

Trine Dale (Norwegian Institute of Fisheries and Aquaculture Research), Sten I Siikavuopio (Norwegian Institute of Fisheries and Aquaculture Research), Anders Aksnes (Norwegian Institute of Fisheries and Aquaculture Research), Britt Hoppe (Norwegian Institute of Fisheries and Aquaculture Research), Mats Carlehög (Norwegian Institute of Fisheries and Aquaculture Research)

Whilst most sea urchin feeds at this point seems capable of promoting gonad growth, there is scope for improvement with respect to texture and taste. This study aims to identify possible common biochemical compounds associated with good (sweet, firm) and bad (bitter, melting) quality in sea urchins, and furthermore how compounds correlated with good quality is influenced by diet. The study is combining sensory analysis with analysis of proximate composition and free amino acids. In part one of the study, three species of wild sea urchins are compared; *Strongylocentrotus droebachiensis* (from Norway and Canada), *S. intermedius* and *Echinus esculentus*. In part two *S. droebachiensis* were fed five diets of different protein and carbohydrate content. In the wild sea urchins there was no apparent relationship between texture and proximate composition. There appear to be a relationship between the intensity of bitter taste and the content of valine and/or the content of alanine and glycine (increased bitterness with increased content of valine and/or reduced content of alanine and glycine). The intensity of sweet taste appears to be related to content of alanine and glycine. The sensory panel identified significant differences in quality between gonads from sea urchins fed different diets. The data from the biochemical analyses of these gonads are not yet analysed.

DIGESTIVE ENZYME ACTIVITY IN BIVALVE TISSUES

Lewis E. Deaton (University of Louisiana at Lafayette)

While the major organ of digestion in bivalve molluscs is the digestive gland, there is direct and indirect evidence that the gills secrete some digestive enzymes. These enzymes may play a role in internal defense, but they may also be secreted to "pre-digest" material that is to be swallowed by the animal. This latter notion is consistent with recent



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research demonstrating that at least in some species of bivalve, the sorting of material trapped on the gills into food to be swallowed and material to be rejected occurs on the gill itself. We have used API ZYM. test kits (bioMerieux) to compare the activity of 19 assorted digestive enzymes in the gill, digestive gland, and adductor muscle of the quahog *Mercenaria mercenaria* and the oyster *Crassostrea virginica*. For most of the enzymes, the activity was highest in the digestive gland and lowest in the adductor muscle. Activities in the gill were intermediate between the former two tissues. Assays are being developed to examine the activity of laminarinase, cellulase, amylase, and proteases in gill, digestive gland and adductor muscle in *M. mercenaria* and *C. virginica*.

RESTORING MERCENARIA ON LONG ISLAND: CONDITION AND SPAWNING OF TRANSPLANTED HARD CLAMS IN SANCTUARIES IN LONG ISLAND, NEW YORK

Michael H. Doall (Stony Brook University), Dianna K. Padilla (Stony Brook University), Carl Lobue (The Nature Conservancy), Laurie Perino (Stony Brook University)

On Long Island, New York, the Nature Conservancy has spearheaded efforts to restore *Mercenaria mercenaria* populations by creating sanctuaries of high densities of spawning clams to reduce sperm limitation for fertilization and thereby increase the potential for recruitment. Success requires that the clams survive, obtain enough energy for gonad development, and spawn. To assess the condition of transplanted clams in sanctuaries established in the Great South Bay and Peconic estuaries, we monitored condition index (CI), computed as the ratio of the dry mass of soft tissue to the internal shell cavity capacity, and qualitative rankings of gonad ripeness during 2004 and 2005. Changes in CI were correlated with changes in gonad ripeness in all sanctuaries monitored; CI increased as gonads ripened and decreased as gonad tissue and gamete concentrations diminished. Decreases in CI and gonad ripeness coincided with the appearance of hard clam larvae in the plankton, indicating that spawning had occurred. The magnitude and timing of changes in CI and gonad ripeness varied between populations of clams in different sanctuary sites, and between years for the same populations. Temperature appeared to be one exogenous factor influencing the timing of reproductive cycles, but spatial-temporal variability in other environmental parameters, such as food quantity and quality, may also explain differences between populations and between years. The prior history of transplanted clams, especially their source location, also appeared to influence condition and timing of gonad development during the first year after transplant.

GEODUCK FARMING 101 - THE TRIALS AND TRIBULATIONS OF STARTING A NEW FARM.

Peter Downey (Discovery Bay Shellfish Inc.)

This presentation will examine issues encountered while planting three acres of geoduck during the 2005 planting season. Issues to be reviewed include securing appropriate tidelands, coming to terms with regulatory agencies and tribes, developing a labor pool, ensuring seed survival, planting logistics and dealing with inclement weather and wind driven waves.

HUSBANDRY PRACTICES AND ITS ASSOCIATIONS WITH PRODUCTIVITY IN PRINCE EDWARD ISLAND MUSSEL FARMS

Andre Drapeau (University of Prince Edward Island), Luc Comeau (Department of Fisheries and Oceans), Jeff Davidson (University of Prince Edward Island)

Associations between husbandry practices and blue mussel (*Mytilus edulis*) productivity in Prince Edward Island (PEI), Canada farms was investigated. Husbandry was measured using SCUBA in Tracadie Bay (2002 – 2004) and in 16 embayment in 2003. Measurements related to 1-year-old crop exposed to approximately eight months of husbandry practices. Between 2002-2004, longline spacing, sock spacing and sock weight in Tracadie Bay ranged from 5 to 24.85 m, 27.50 to 40.40 cm and 2.86 to 15.49 kg respectively, while across PEI husbandry ranged from 1.50 to 29.54 m, 26.38 to 62.40 cm and 1.48 to 16.87 kg respectively. Sock spacing significantly increased by 32% (+12 cm) over the years and was directly responsible for significantly decreasing lease stocking density by 25% (-6

socks/100 m²). Regression models revealed that sock spacing was positively associated with mussel sock weight in Tracadie Bay (2002) and across PEI (2003). In Tracadie Bay, sock spacing was related to sock weight in 2002 and mussel condition in 2004. Each additional 10 cm between socks resulted in raising predicted sock weight by 1.15 kg and mussel condition index by 1.60, while across PEI, sock weight increased 0.74 kg. This gain is plausible and likely driven by reduction in intraspecific competition between mussels for available food resources. However, the observed correlation was statistically significant only for a single year in Tracadie Bay (2002), while marginally significant across PEI (2003). This suggests that the effect of sock spacing was dynamic and dependent upon food availability within the water column.

OYSTER SURVIVAL AND GROWTH IN THE BARATARIA ESTUARY

Ronnie Duke* (Department of Biological Sciences, Nicholls State University), Earl J. Melancon (Department of Biological Sciences, Nicholls State University)

We document oyster responses to natural environmental events in the historically productive zone of the Barataria estuary, part of the Davis Pond Mississippi River Diversion outfall area. The diversion has operated minimally with no measurable impact on oysters. During the study there were two naturally occurring reduced-salinity events (<5ppt), two tropical storms and three hurricanes.

Oyster populations were studied monthly from April 2004 to November 2005 for survival, size frequency and condition index. In June/July '04 salinities were well below 5ppt across most of the estuary and all oysters died except at the most down-estuary site. Oysters rebounded with a good estuary-wide spat set in September/October '04. All fall '04 set oysters survived an estuary-wide winter '05 reduced-salinity event. Oysters exhibited highest condition indices during winter and lowest during summer.

Seed-sized oysters, @44mm in shell length, were tagged in fall '04 at two sites for monthly growth comparisons. From September '04 to August '05 oysters grew to 64.3±6.9mm at the up-estuary site and to 77.5±7.3mm at the down-estuary site. A shell length of 65mm is minimum sack size acceptable for oysters harvested from private leases. Preliminary comparisons of the fall '04 sets at 10 sites for size frequency exhibited a similar pattern of faster growth down-estuary.

This information becomes important for natural resource managers and oystermen as adaptive management strategies are developed so that both diversions and oysters can co-exist. Oystermen must decide if they want to harvest seed-size oysters or wait the additional time to harvest sack-sized oysters.

PERKINSUS OLSENI IN VITRO ISOLATES FROM NEW ZEALAND CLAMS, AUSTROVENUS STUTCHBURYI

Christopher F. Dungan (Maryland Dept. of Natural Resources, Oxford Laboratory), Rosalee M. Hamilton (Maryland Dept. of Natural Resources, Oxford Laboratory), Kimberly S. Reece (Virginia Institute of Marine Science), Jessica A. Moss (Virginia Institute of Marine Science), Ben K. Diggles (DigsFish Services Pty Ltd, Queensland, Australia)

Prevalent *Perkinsus* sp. infections have been reported in *Austrovenus stutchburyi* venerid clams (cockles) from northern New Zealand, since 2000. Sequencing of rRNA gene complex ITS- and NTS-regions of genus-*Perkinsus* PCR amplicons from infected *A. stutchburyi* DNA templates, have consistently indicated the presence of *P. olseni* parasites. However, in vitro *Perkinsus* sp. isolates have been unavailable to date for comprehensive morphological and genetic evaluation of the New Zealand parasite's identity. We report in vitro isolation, propagation, cloning, and cryopreservation of four *Perkinsus* sp. isolates from a 2005 sample of *A. stutchburyi* cockles from northern New Zealand's Mangamangaroa Stream (24 °C, 32 ppt). Isolates were propagated in vitro despite a moderate prevalence (24%) of low-intensity *Perkinsus* sp. infections in the sampled cockles. These isolates showed morphometric similarities and differences with characteristics reported for *P. olseni* isolates from other hosts and locations. Although their frequency of continuous in vitro zoosporulation was low, one typical isolate was induced to wholesale (> 70%) zoosporulation by a transient, 48-h passage through ARFTM. Nucleotide sequences of rDNA ITS-regions from our isolates consistently grouped them with *P. olseni* sequences in phylogenetic analyses, suggesting that *Perkinsus* sp. infecting New Zealand *A. stutchburyi* clams are conspecifics of destructive and



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broadly distributed *P. olseni* parasites of several eastern Asia clams, diverse Australian clams and abalone, and several European clams.

WASHINGTON STATE GEODUCK FISHERY MANAGEMENT

Sarah Dzinbal (Washington State Department of Natural Resources), Todd Palzer (Washington State Department of Natural Resources)

The Washington State Department of Natural Resources (DNR), as the steward of publicly owned lands, manages approximately 2.4 million acres of aquatic lands in Washington. One of its roles is to co-manage the wild stock geoduck fishery, and the agency does this together with the Washington Department of Fish and Wildlife (WDFW) and the Treaty Tribes in western Washington. The geoduck fishery has been open since 1970 and is managed to be sustainable for future generations of citizens in Washington State. The state and tribes work collaboratively to quantify geoduck biomass by conducting tract surveys on a rotational basis. WDFW sets the Total Allowable Catch for each fishery year—2.7% of the total commercial geoduck biomass—and the state and tribes share this resource equally. DNR publicly auctions the right to harvest the state's share of the geoduck resource, in the form of geoduck quotas. This generates approximately \$8 million per year for the state, which is used by a number of state and local government entities for aquatic land enhancement and management work. The successful bidders at auction sign harvest agreements with DNR; the agreements allow companies to harvest specified pounds of geoducks from specified tracts of state owned aquatic lands for a specified period of time. The agreements are enforced by DNR's commercial dive team, which conducts daily fishery compliance operations on the geoduck tract every day the state fishery is open. The fishery is a year-round dive fishery.

WASHINGTON STATE GEODUCK AQUACULTURE PROGRAM

Sarah Dzinbal (Washington State Department of Natural Resources), Celia Barton (Washington State Department of Natural Resources), Jeffrey Schreck (Washington State Department of Natural Resources)

The 2003 Washington State Legislature directed the Washington State Department of Natural Resources (DNR) to determine the feasibility of geoduck aquaculture on state owned aquatic lands (SOAL). Between July 2003 and June 2005, DNR contracted with the University of Washington and other entities to compile reports that would allow that determination to be made. DNR testified to the 2005 Legislature that geoduck aquaculture on SOAL is feasible, but recommended that the project proceed with caution by authorizing the use on twenty to twenty-five acres of SOAL per year. DNR submitted a budget request for additional funds to implement a leasing program for geoduck aquaculture on SOAL, but unfortunately did not receive those funds. DNR believes that aquaculture is an important use and, as such, has moved forward with implementation of a geoduck aquaculture-leasing program. The lack of dedicated funding has necessitated a different approach—DNR is designing a monitoring program that will require some of its geoduck aquaculture lessees conduct scientific monitoring on their leaseholds throughout the first geoduck growing cycle. The results of this monitoring will allow DNR to fully assess the environmental effects of geoduck aquaculture, and further allow a determination on the long-term feasibility of this use. Twenty to twenty-five acres of SOAL will be put up for competitive bid in the summer of 2006. Potential lessees will respond to the Request for Proposals by providing a summary of their proposed activities, including the scientific monitoring aspect.

SURVIVAL AND GROWTH OF CRASSOSTREA VIRGINICA LINES IN CHESAPEAKE AND DELAWARE BAYS.

Lionel Dégremont (Virginia Institute of Marine Science), Standish K. Allen Jr (Virginia Institute of Marine Science), E.M. Burreson (Virginia Institute of Marine Science), Gregory Debrosse (Haskin Shellfish Research Laborator)

As a part of our oyster breeding program developed to enhance resistance against MSX and Dermo in *Crassostrea virginica*, eight lines and one control were produced in 2004 and deployed at four sites in Virginia (Burton's Bay, York River, Kinsale and Lynnhaven) and one site in the Delaware Bay (Cape Shore). First results, excluding the Cape Shore site, showed difference of mortality among sites in November 2005 with the highest mortality at Burton's Bay (57%), intermediate at the York River and Lynnhaven sites (40-46%) while oysters at Kinsale showed the lowest mortality (10%). The mortality mostly occurred between August and November except at Burton's Bay where it happened in April. Significant differences of mortality were also observed among lines in each site and a significant line by environment interaction was found which is easily explainable by the absence of significant mortality at Kinsale and the unusual mortality event occurring in April at the Burton's Bay. For growth, oysters deployed at York River and Lynnhaven showed the slowest growth while those at Kinsale and Burton's Bay showed the fastest. Finally, our results suggested which line would be most useful according to their yields across environments. For example in Burton's Bay, CROSBreed line yielded 2.5 as much as the control while at Kinsale, a Louisiana strain would be preferred, yielding 60% greater than the control. Pathology analyses indicate MSX explains the mortalities observed during the summer and results from the fall sampling will provide more perspective on the cause of the mortalities.

COMPARISONS OF POST-SETTLEMENT SURVIVAL AND GROWTH IN CRASSOSTREA VIRGINICA AND C. ARIAKENSIS IN RELATION TO TIDAL EMERSION.

Peterks@vims. Edu (Virginia Institute of Marine Science), M. Luckenbach (Virginia Institute of Marine Science)

In many high salinity regions of the mid-Atlantic *C. virginica* is limited to the intertidal zone where it achieves a partial refuge from predation. Persistence in such intertidal habitats necessitates tolerance of both desiccation and extremes of temperature during periods of aerial exposure. Such capabilities in *C. ariakensis* have yet to be determined.

The effects of aerial exposure by tidal emersion on comparative survival and growth rates of diploid *C. ariakensis* and diploid *C. virginica* were investigated using oysters set on plastic tiles and grown in a flow-through quarantine system. Four tidal regimes were simulated; 1) *high intertidal* (3.5 hrs emersion), 2) *mid tide* (2 hrs emersion), 3) *low intertidal* (1 hr emersion) and 4) *subtidal* (constant immersion). Vertical ("North-facing" and "South-facing") and horizontal ("Up" and "Down") tile orientation treatments were also incorporated in the experimental design. Tiles were individually photographed on a weekly basis between June and August 2005 and image analysis software was used to gather survival and growth data.

The high intertidal treatment exceeded the physiological tolerances of both species; complete mortality occurred within the first 2 weeks. In the mid tide and the low intertidal treatments *C. ariakensis* and *C. virginica* exhibited intermediate growth and survival. Growth rates were highest and levels of mortality were lowest in the subtidal treatment. Determination of the tolerance of *C. ariakensis* to aerial exposure will improve our understanding of its potential to colonize intertidal habitats, to compete with native species for resources, and to become a fouling nuisance if introduced.

USE OF THE OLIGOHALINE CLAM RANGIA CUNEATA AS A BIVALVE INDICATOR OF WATERSHED ALTERATIONS IN SOUTHWEST FLORIDA.

Vincent Encomio (Coastal Watershed Institute - Florida Gulf Coast University), Christina Panko (Coastal Watershed Institute - Florida Gulf Coast University), Ernest Estevez (Center for Coastal Ecology - Mote Marine Laboratory Sarasota), Aswani K. Volety (Coastal Watershed Institute - Florida Gulf Coast University)

In Florida, freshwater releases from Lake Okeechobee result in dramatically lowered salinity, adversely affecting bivalve populations downstream in the Caloosahatchee River. Additionally, high sediment loading due to increased freshwater flow or dredging may adversely affect bivalve condition. The impact of watershed alterations on oysters is part of ongoing research in SW Florida. The use of oysters as a sentinel organism, however, only covers the mesohaline (10-30 parts per thousand (ppt)) regions of the estuary. Few, if any studies have examined effects on bivalves in the oligohaline (<10 ppt) portions of the Caloosahatchee estuary. The clam *Rangia cuneata* is found extensively in oligohaline regions of the Caloosahatchee and may be a useful bivalve indicator of sedimentation and

altered salinity. Clams were exposed for thirty days to clay (< 4 μM grain size) and silt (40-60 μM) at 0, 1 and 2 grams/clam/day. At 0, 10, 20 and 30 days condition index (dry tissue weight:dry shell weight), glycogen, lipid and protein content in clams were measured. Condition index decreased significantly over time ($p=0.0177$) but not with sediment type and dose. Despite wide physiological salinity tolerances, *R. cuneata* cannot maintain stable populations beyond 1-15 ppt (Hopkins et al. 1973), making it vulnerable to extended freshets and droughts. Distributions of live and dead shells indicate higher proportions of dead shells near the head of the estuary, possibly indicative of past freshets. Salinity tolerances and responses to interactive stress (e.g. temperature) will be examined in the lab to corroborate patterns seen in the wild.

COMMUNITY SHIFT ASSOCIATED WITH SHELLFISH AQUACULTURE IN TWO MID- ATLANTIC ESTUARIES

Patrick Erbland (Delaware State University), Gulnihal Ozbay (Delaware State University)

Populations of the eastern oyster (*Crassostrea virginica*) and its associated fishery have been declining in the State of Delaware and greater Mid-Atlantic region of the United States for decades. This shortage of oysters continues despite intensive husbandry of oyster reefs by fishers and government agencies. Consequently there is interest in the culture of *C. virginica* enclosed in “grow out gear” (GOG) to increase yields.

Such a practice has a multitude of associated impacts on the host ecosystems.

These include reduced nutrient load in the water column (Ulanowicz and Tuttle 1992) and enrichment of benthic sediments (Nugues et al 1996). The enhanced levels of sport fish such as Striped Bass (*Morone saxatilis*) and Bluefish (*Pomatomus saltatrix*) found on oyster reefs (Harding and Mann 1998) may also exist on oyster farms.

This is a two part study investigating shifts in the benthic and infaunal communities. We will compare the diversity and abundance of species inhabiting subtidal “Rack and Bag” type GOG, containing *C. virginica*, with an adjacent, created *C. virginica* reef in Indian River Bay, DE. Secondly, we will compare the diversity and abundance of infaunal species present below intertidal oyster gear with an adjacent control area of open sand flat on the eastern shore of Delaware Bay.

This study will provide insight into the ecological impact of shellfish aquaculture and be useful in incorporation of *C. virginica* aquaculture into the management schemes of concerned regulatory agencies.

ADDITIONAL EVIDENCE OF HIGH RESISTANCE TO HAPLOSPORIDIUM NELSONI (MSX) IN THE NATIVE OYSTER POPULATION OF DELAWARE BAY.

Susan Ford (Haskin Shellfish Research Laboratory, Rutgers), David Bushek (Haskin Shellfish Research Laboratory, Rutgers)

Natural selection during the first *Haplosporidium nelsoni*-caused epizootic in Delaware Bay oysters resulted in the development of a degree of resistance to mortality caused by the pathogen throughout the Bay. Until the late 1980s, this level of resistance did not change because most of the surviving oysters were protected from additional selection as they resided in the low salinity region of the Bay. The development of a second level of resistance was signaled after heavy *H. nelsoni*-caused mortalities in the mid 1980s occurred during a severe drought and prevalence of the pathogen subsequently declined markedly. Susceptible controls exposed beside natural set in the lower bay continued to become heavily infected, whereas the natural set had prevalences of 0 to 10%. To determine whether oysters on the low-salinity beds were similarly resistant, we exposed oysters from two upper-bay beds along with lower bay natives and susceptible controls in Cape May Harbor where *H. nelsoni* pressure has been heavy. Six months later, prevalence of susceptible oysters was 90%, with most infections being advanced. At the same time, prevalence in the two upper bay groups was only 5 to 20% with mostly light, localized infections. This confirms that a high degree of resistance is now present throughout the Bay. In contrast, there is little evidence that significant

resistance to infection by *Perkinsus marinus* has developed despite continuous exposure and consequent mortality over the past 15 years.

COMPARATIVE EFFECTS OF NEUROTRANSMITTERS AND CALCIUM BLOCKERS ON ISOLATED GILLS AND WHOLE ANIMALS OF THE MUSSEL, MYTILUS EDULIS

Dana M. Frank (University of Connecticut), J. Evan Ward (University of Connecticut), Sandra E. Shumway (University of Connecticut), Lewis Deaton (University of Louisiana at Lafayette)

It is generally accepted that the lateral cilia of bivalve molluscs are inhibited by dopaminergic systems and excited by serotonergic systems. Most of this experimentation has been carried out on excised gills of the mussel, *Mytilus edulis* and not on whole animals. We conducted preliminary tests to compare the effects of the neurotransmitters serotonin (5HT), dopamine (DA), and apomorphine and the calcium channel blocker lanthanum on pumping activity of intact *M. edulis* and the ciliary activity of isolated gill tissue. We also exposed isolated gill filaments to the calcium channel blockers cobalt chloride and verapamil. Whole animals and freshly excised gill sections were kept in filtered seawater at ambient temperature (~ 20 °C). Experimental drug was added directly to the bath water. Activity of the lateral cilia of isolated gills was monitored with an inverted compound microscope. Water processing rates of whole animals were examined using particle image velocimetry (PIV). Lanthanum induced shedding of the gill epithelia in excised sections and in whole animals obviating any effect on the lateral cilia. Cobalt chloride, DA, and verapamil had no effect on gill sections. When exposed to 1×10^{-6} M apomorphine, 30% of gill sections showed some inhibition; 50% were inhibited at 1×10^{-5} M. In the whole animal, 1×10^{-6} M apomorphine had a significant negative effect on velocities of particles in the excurrent flow. 5HT appeared to increase synchrony (metachronism) of the lateral ciliary beat in isolated gill sections. Preliminary work suggests that neural agents affect whole animals differently than isolated gill tissue.

OPTIMIZATION OF OXYTETRACYCLINE TREATMENT IN TWO ABALONE SPECIES, HALIOTIS SORENSENI AND H. RUFESCENS

Carolyn S. Friedman (School of Aquatic and Fisheries Sciences, Univ. Washington), B.B. Scott (School of Aquatic and Fisheries Sciences, Univ. Washington), R.E. Strenge (School of Aquatic and Fisheries Sciences, Univ. Washington), N.A. Wight (School of Aquatic and Fisheries Sciences, Univ. Washington), Thomas B. McCormick (Channel Islands Marine Research Institute, Port Hueneme, CA), George Trevelyan (The Abalone Farm, Inc, Cayucos, CA)

Withering syndrome (WS), a rickettsial disease, causes losses of wild and farmed abalone. The endangered white abalone, *Haliotis sorenseni*, is highly susceptible to WS and information on management of this disease is essential for successful restoration. White abalone were fed a medicated diet containing oxytetracycline (OTC) for 20d; digestive gland (DG) and foot muscle (FM) were assayed for OTC and rickettsia at days 3, 18, 24, 40, 67, 80, 110, 129, 165 and 185 post-medication. Abalone were re-challenged with rickettsia at 24, 40, 67, 146 and 171d. DG contained higher levels and retained OTC longer than FM. Mean DG drug levels peaked at 996ppm at 25d post-medication and leveled off at 30ppm after 68d. FM peaked at 18ppm and declined to below 2ppm, the FDA tolerance level, between 25 and 41d. Protection from rickettsial infections occurred in abalone with over 50ppm in the DG.

We investigated the pharmacokinetics of one oral OTC dose in red abalone and that of 1, 2 or 3 intramuscular (IM) injections with Liquamycin-LA 200 in white abalone. One oral dose cleared 90-100% ($p < 0.05$) of infections. A mean of 239ppm OTC was detected in the DG 5d after medication followed by depletion to 2.34ppm after 130d. In a second trial, 688ppm accumulated in the DG at 1d and declined to 326ppm by day 5. Interestingly, total body OTC was only 89ppm at day 1 (688ppm in DG). IM injections resulted in DG accumulations of 7.15, 20.3 and 27.4ppm after 1, 2 and 3 doses, respectively.

DEVELOPMENT AND APPLICATION OF TYPE I MARKERS FOR LINKAGE MAPPING AND POPULATION GENETICS IN CRASSOSTREA SPECIES

Patrick Gaffney (University of Delaware), Hyungtaek Jung (University of Delaware), Woo-jin Kim (National Fisheries Research & Development Institute), Robin Varney (University of Delaware), Coren Milbury (University of Delaware)

In oysters, nuclear markers developed for linkage mapping and the analysis of population structure include allozymes, anonymous single copy DNA (scnDNA) loci, microsatellites, and most recently, single nucleotide polymorphisms (SNPs). The emergence of substantial expressed sequence tag (EST) databases for *Crassostrea virginica* and *C. gigas* has enabled researchers to probe candidate genes for polymorphisms by direct DNA sequencing. Because the number and locations of introns in genes selected from EST databases are unknown, primer design for amplification from genomic DNA can be challenging. Taking advantage of the reduced frequency of introns in the 3'UTR of invertebrate genes, we have designed primers for small (~300 bp) amplicons spanning the 3' end of coding sequence and the 3'UTR. These primers typically provide successful amplifications and often amplify putatively homologous sites in closely related *Crassostrea* species. High levels of polymorphism ensure that even short amplicons usually have one or more SNPs suitable for genotyping. Applications of markers developed to date will be discussed, including linkage mapping in *C. virginica* and *C. gigas*, population genetics and taxonomy of Atlantic and Asian oysters, and monitoring restoration efforts involving hatchery-produced seed.

REPRODUCTIVE TIMING AND SUCCESS OF FRESHWATER MUSSELS

Heather S. Galbraith (Oklahoma Biological Survey and the University of Oklahoma), Caryn C. Vaughn (Oklahoma Biological Survey and the University of Oklahoma), Daniel E. Spooner (Oklahoma Biological Survey and the University of Oklahoma)

Freshwater mussels are among the most imperiled groups of aquatic organisms on earth. Their diversity, dominance in benthic biomass, and unique life histories make them important contributors to stream ecosystem function while at the same time have made them susceptible to anthropogenic interferences such as river regulation, impoundments, and global warming. High species richness and large numbers of closely related species within mussel beds has proven paradoxical to typical views of speciation in broadcast spawning organisms. The goal of our research is to determine the reproductive timing, success, and isolation of three closely related species of freshwater mussels by addressing the following questions: 1. What are the mechanisms of reproductive isolation in freshwater mussels? 2. How is reproductive timing regulated and important to fertilization success in freshwater mussels? In order to conserve mussel communities, understanding the processes of speciation and reproduction are vital. This work will be some of the first to provide an evolutionary and ecological perspective on one of the most critical and little understood periods of the freshwater mussel life cycle, specifically that prior to fertilization.

GREEN CRABS (CARCINUS MAENAS) AS THE GRIM REAPER: DESTRUCTION OF EELGRASS BEDS IN NOVA SCOTIA

David J. Garbary (Department of Biology, St. Francis Xavier University), A. G. Miller (Department of Biology, St. Francis Xavier University)

Following the first appearance of invasive *Carcinus maenas* in the southern Gulf of St. Lawrence in the early 1990s, 385,000 crabs km⁻² occurred in Antigonish Harbour in 2000. Eelgrass (*Zostera marina*) which had large stable populations in the estuary for the previous 60 years declined by 95 % in 2001, and many parts of the harbour were devoid of living eelgrass. This decline also occurred in adjoining estuaries where large numbers of crabs also occurred, but did not take place in estuaries further west and north where green crabs had not yet invaded, or numbers were low. In July-August 2002, shoot density of eelgrass at an experimental site in Tracadie Harbour with high crab numbers declined by 75% when a control harbour with few crabs showed increasing shoot density. A field experiment using 1.5 m² cages, with and without green crabs, suggested that *C. maenas* was responsible for the loss of eelgrass. Telephone interviews with knowledgeable observers around Nova Scotia also suggested a correlation between declining eelgrass populations and increasing numbers of crabs during the previous ten years. The impact of green crabs on eelgrass is associated with their foraging activity in which they produce pits up to 30 cm wide and

15 cm deep. During foraging, eelgrass shoots are dislodged by crab digging, or the shoot bases are shredded, causing release of whole shoots into the drift. Since 2001, green crab numbers have been in decline in Antigonish Harbour, and there has been a substantial recovery of *Z. marina*.

DETECTION AND DISTRIBUTION OF QUAHOG PARASITE UNKNOWN IN THE COASTAL MARINE ENVIRONMENT

R. J. Gast (Woods Hole Oceanographic Institution), D. M. Moran (Woods Hole Oceanographic Institution), K. R. Uhlinger (Marine Biological Laboratory), D. R. Leavitt (Roger Williams University), R. Smolowitz (Marine Biological Laboratory)

Quahog Parasite Unknown (QPX) is a significant cause of hard clam mortality along the Northeast coast of the United States. The QPX organism is classified as a thraustochytrid protist, and members of this protistan family are generally saprophytic and commonly found in the marine environment. No viable method was available to reliably survey the natural environment for QPX, to rapidly assess the persistence of the organism in previously affected plots, and to screen large numbers of clams potentially exposed to the disease. Here we report on a PCR-based method that we have developed that permits sensitive detection of QPX in natural samples and seed clams. With our method, between 10-100 QPX cells can be detected in 1L of water, 1 gram of sediment and 100mg of clam tissue. We have used the method to examine one hundred 15 mm seed clams, and found that 10-12% of the clams were positive for the presence of the QPX organism, although only 1% showed histological evidence of infection. The method has also been used in a survey for the presence of the pathogen in environmental samples from a site experiencing severe infection and a site with low to no infection. This survey has revealed the presence and year-round persistence of QPX in the environment, even in the absence of large-scale die-off.

CONDITION OF FRESHWATER MUSSELS HELD IN REFUGIA AT THE WHITE SULPHUR SPRINGS NATIONAL FISH HATCHERY, WEST VIRGINIA.

Catherine M. Gatenby (United States Fish and Wildlife Service), Matthew A. Patterson (United States Fish and Wildlife Service), Julie L. Devers (United States Fish and Wildlife Service), Danielle A. Kreeger (Delaware River Basin Commission)

The USWFS established a refugium at the WSSNFH for endangered freshwater mussels residing in harms way of a bridge replacement in the Allegheny River. Approximately 600 common mussels from 2 species sharing a similar reproductive strategy as target endangered species were salvaged in October, 2004; endangered mussels were salvaged in May 2005 as well. In August 2005, 450 common mussels were relocated to WSSNFH, and were included in a diet quality feeding study. Non-lethal and lethal measures of physiological activity and condition are used to monitor condition of common mussels.

After one year in captivity, short-term brooders have maintained condition and survival better than long-term brooders. Condition of *Elliptio dilatata* was not significantly different than wild *E. dilatata* in September 2005; whereas, captive *Actinonanias ligamentina* condition was significantly less than their wild counterparts.

Endangered *Pleurobema clava* had higher survival (100%) than endangered *Epioblasma. t. rangiana* (8%). Similarly, *E. dilatata* (74%) had higher survival than *A. ligamentina* (66%). Mussels collected in October 2004 were unusually low in physiological condition, and were likely stressed by high water conditions and high suspended bed loads in the Allegheny River throughout most of 2003 and 2004. WSSNFH modified culture systems in May 2005 to improve food delivery and food quality. Survival of endangered mussels brought into captivity in spring 2005 ranges 93%-100% for *E. t. rangiana* and *P. clava*. Captive care protocols developed from this effort will be available as conservation tools in the future for mitigating loss of a resource due to in-stream activities.



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TAQMAN© MGB REAL-TIME PCR APPROACH TO QUANTIFICATION OF PERKINSUS MARINUS AND PERKINSUS SPP. IN OYSTERS

Julie D. Gauthier (University of North Carolina, Center for Marine Science), Chris R. Miller (Applied Biosystems), Ami E. Wilbur (University of North Carolina, Center for Marine Science)

Several molecular diagnostic assays have been developed in an attempt to replace the traditional Ray's Fluid Thioglycollate Medium (RFTM) assay for detection and quantification of *Perkinsus marinus* in oysters. Real-time PCR technology is now the state-of-the-art method currently used to diagnose disease intensity in vertebrates. We developed a simple (two-reagent) real-time PCR assay to quantify *P. marinus* (PMAR) and *Perkinsus* spp. (PERK) in oysters, using TaqMan© assays designed with Minor Groove Binder (MGB) probes on an Applied Biosystems 7500 Real-Time PCR System. This approach addresses several factors that can interfere with optimal real-time PCR performance. Both PERK and PMAR assays demonstrate strong correlations ($R^2 \geq 0.99$) between parasite cell density and real-time PCR threshold cycle (C_T) with amplification efficiencies $\geq 99\%$. The PERK assay results in similar amplification plots for the three species tested (*P. marinus*, *P. atlanticus* and *P. andrewsi*), whereas the PMAR assay reported only *P. marinus*. A strong correlation ($R^2 > 0.90$) was found between infection level determined by the traditional RFTM method and quantification by real-time PCR based on internal standards prepared from *P. marinus* spiked oyster tissue. The PCR assays also detected false negatives (15%) diagnosed by the traditional method. We demonstrate the usefulness of these assays in replacing the traditional method for a non-subjective, sensitive, specific and accurate quantification of *Perkinsus* spp. in oyster tissue, with particular application in analyzing frozen or archived material.

IS HABITAT AVAILABILITY LIMITING RECRUITMENT OF CALICO SCALLOPS (ARGOPECTEN GIBBUS)?

Stephen P. Geiger (Florida Fish & Wildlife Research Institute), Janessa C. Cobb (Florida Fish & Wildlife Research Institute)

The Florida calico scallop fishery has declined from a peak of 42.7 million pounds of adductor muscle meats landed in 1984 to none landed in 2005. Removal of scallop and other shell as bycatch may have depleted the essential fisheries habitat for settling veligers. The goal of this study was to describe current abundance and distribution of scallops, shell, and associations between spat and its preferred substrate on two of the historic scallop fishing grounds.

Scallops were collected at 31% of the Atlantic stations, spat at 9%, and both calico scallop shell and other shell at 89%. Spat were most commonly found on scallop shells (46%), but were also found loose, on other shells, rocks and trash. Shell from other molluscs was 1.75 times as abundant as scallop shell. Potential predators that were abundant included *Astropecten articulatus*, *Portunus gibbesii* (also common in the Gulf) and *Distorsio clathrata*.

Scallops were collected at 10% of the Gulf of Mexico stations, spat at 8%, scallop shell at 63%, and other mollusc shell at 71%. Rocks and hard bottom were common. Spat preferred other mollusc shells (64%) but were also found loose, on rocks, and on calico scallop shells. Shell from other molluscs was 20 times as abundant as scallop shell.

Our findings suggest that the Cape Canaveral calico scallop bed currently has a similar spatial extent to historic records, and that calico scallops are seasonally abundant and are associated with shell deposits. The southwest Florida scallop bed is limited in extent and abundance.

INGESTION RATES AND LARVAL DEVELOPMENT ON NATURAL AND ARTIFICIAL PARTICLES

Sophie B. George (Biology department, Georgia Southern University)

Ingestion rates and growth to metamorphosis for five species of echinoderm larvae were investigated in the laboratory using artificial and natural diets. The natural algal diets included *Dunaliella tertiolecta*, *Isochrysis galbana* and *Rhodomonas* sp., and the artificial diet used was a 10-fold diluted artificial feed made from Ziegler E-Z larval diet. The volume of cells ingested varied significantly among species, diets and larval age. All echinoderm larvae ingested significantly higher volumes of the larger alga *Rhodomonas* and the medium-sized alga *Dunaliella* than the artificial diet. Younger larvae ingested large and medium-sized particles at lower rates than older larvae. *Dendraster* larvae were more efficient at ingesting both the large and small particles and less efficient in ingesting the medium-sized particles compared to *Strongylocentrotus* larvae. The differences in ingestion rates among species might be due to differences in size, form, water velocity over the ciliated band, density of cilia on the ciliated larval band, and differences in size and palatability of the various diets. Though less of the artificial diet was ingested, *Lytechinus* and *Dendraster* larvae developed to metamorphosis at similar rates and attained similar sizes to those fed *Dunaliella* or the mixed algal diet of *Isochrysis galbana* and *Dunaliella*. An artificial feed would eliminate the need to culture phytoplankton and provide the potential of establishing nutritional requirements for echinoderm larvae. Increasing the size of the artificial particles to 300µm for younger larvae and 600µm for older larvae might lead to optimal ingestion rates and the production of large juveniles at metamorphosis

THE EFFECTS OF DIETARY CHOLESTEROL AND PHOSPHOLIPIDS ON GROWTH AND PRODUCTION IN THE SEA URCHIN *LYTECHINUS VARIEGATUS*

Victoria K. Gibbs (Univ. of Alabama at Birmingham), Stephen A. Watts (Univ. of Alabama at Birmingham), Addison L. Lawrence (Texas A&M University System), Mickie L. Powell (Univ. of Alabama at Birmingham), Hugh S. Hammer (Univ. of Alabama at Birmingham), Warren T. Jones (Univ. of Alabama at Birmingham), John M. Lawrence (Univ. of South Florida)

Lipids are important components in the diets of marine organisms. In this study, the effects of dietary cholesterol and phospholipids were investigated for small *Lytechinus variegatus* to determine a requirement for either nutrient. *Lytechinus variegatus* (n=16; average initial wet weight =13.9 ± 0.15g) were fed semi-purified formulated feeds supplemented to produce low, medium, or high levels of cholesterol (supplemented with 0, 0.3, or 0.6%, as fed) or phospholipids (supplemented at 0, 3, or 4%, as fed) for 12 weeks. Experimental levels of cholesterol and phospholipids were based on established requirements for marine invertebrates. Under the conditions of this study, dietary cholesterol did not have a significant effect on weight gain or gut or gonad production, although total production efficiency was lowest ($P=0.007$) for individuals fed the low cholesterol feed (36.0, 47.6, and 41.9% for low, medium, and high levels, respectively). Individual weight gain and gonad production were highest for individuals fed the high phospholipid feed (final wet weight =38.6, 40.3, and 45.8g, $P<0.001$; gonad wet weight =5.3, 6.2, and 6.8g, $P<0.05$, for low, medium, and high levels, respectively). Total production efficiency was lowest ($P=0.001$) for individuals fed the low phospholipid feed (35.1, 47.6, and 45.6%, respectively). However, organ production was not substantially affected by cholesterol or phospholipids when adjusted for total individual wet weight (ANCOVA $P>0.05$). These data suggest that supplemental dietary cholesterol and phospholipids support weight gain in *Lytechinus variegatus*. This study was supported by the Mississippi-Alabama Sea Grant Consortium.

OPTIMIZING METHODS FOR DETECTION OF CRYPTOSPORIDIUM OOCYSTS IN NATIVE OYSTERS FROM THE CHESAPEAKE BAY

Autumn Girouard (Johns Hopkins Bloomberg School of Public Health), Thaddeus Graczyk (Johns Hopkins Bloomberg School of Public Health)

Several groups have documented the presence of *Cryptosporidium*, an anthroponozoonotic enteric parasite, in oysters harvested for commercial purposes. Getting accurate estimates of *Cryptosporidium* contamination levels in oysters is difficult because recovery efficiencies are dependent on the isolation method used. Such estimates are important for determining the human health risks posed by consumption of contaminated oysters in a raw form. In this study, recovery efficiencies are compared for multiple methods used to isolate *Cryptosporidium* oocysts from experimentally contaminated oysters. Oysters were harvested after being allowed to filter oocysts from

spiked aquarium water for approximately 24 hours. In one group, the entire oyster meat and all hemolymph was processed by diethyl ether extraction followed by immunomagnetic separation (IMS) of oocysts from the resulting pellet. In another group, the oysters were dissected so that only gills, digestive diverticula and hemolymph were processed as described above. In the last group, oocysts were isolated directly from hemolymph alone by IMS. Recovered oocysts were enumerated by IFA. Recovery efficiencies for the methods described above were also determined using oyster tissue homogenate and hemolymph spiked with 1000 oocysts, to control for differences in recoveries related to differences in oocyst uptake rates among oysters. The results from this study will be used to determine the best method for quantifying *Cryptosporidium* levels in oysters collected from the Chesapeake Bay. This method would have broad application to native and non-native commercially harvested oysters and would be beneficial for regulatory agencies charged with determining safety of shellfish for human consumption.

INVESTIGATIONS INTO SOME EARLY-LIFE HISTORY STRATEGIES FOR CARIBBEAN SPINY LOBSTER AND IMPLICATIONS FOR PAN-CARIB CONNECTIVITY

Jason S. Goldstein (University of New Hampshire), Mark J. Butler IV (Old Dominion University), Hirokazu Matsuda (MPFRC, Japan)

Worldwide, spiny lobsters possess extended larval periods that can easily exceed six months. During this protracted early-life history, pelagic larvae that are produced in one locality disperse throughout oceanic waters, and later settle in far-flung locations to grow and eventually support fisheries in their new home. Observations and limited empirical evidence suggest however that active behavior for Caribbean spiny lobster (*Panulirus argus*) larvae (phyllosoma) and postlarvae (pueruli) appears to have a significant impact on distribution and settlement. In support of this hypothesis, a series of laboratory studies were undertaken to more fully elucidate aspects of phyllosoma and pueruli responses to two selected cues, light and chemical cues, respectively. Phyllosoma culture experiments were initiated to quantify stage duration to the puerulus stage ($>175 \pm 30$ days after hatch (DAH)) as well as stage-specific differences (vertical movements) in response to simulated oceanic light. Two clear behavioral phototactic shifts to light were observed at ca. 60 and ca. 130 DAH using a custom-made light chamber. A complimentary series of laboratory experiments were carried out to test postlarval orientation and the potential for metamorphic delay in response to chemical cues characteristic of the nearshore nursery environment. Using field-caught animals, results showed that lobster postlarvae are attracted to coastal water as well as to the metabolites of red macroalgae ($n \approx 300$ trials) in laminar flow chambers. These findings coupled together enhance our understanding of the transport and settlement processes operating for this species throughout the Caribbean and will help to further validate oceanographic lobster models whose management implications are far reaching.

RECOVERY, BIOACCUMULATION, AND INACTIVATION OF HUMAN WATERBORNE PATHOGENS BY *CRASSOSTREA ARIAKENSIS*

Thaddeus K. Graczyk (Johns Hopkins Bloomberg School of Public Health, Baltimore), Autumn S. Girouard (Johns Hopkins Bloomberg School of Public Health, Baltimore), Leena Tamang (Johns Hopkins Bloomberg School of Public Health, Baltimore), Sharon P. Nappier (Johns Hopkins Bloomberg School of Public Health, Baltimore), Kellogg J. Schwab (Johns Hopkins Bloomberg School of Public Health, Baltimore)

Introduction of non-native oysters (i.e., *Crassostrea ariakensis*) into the Chesapeake Bay has been proposed as necessary for restoration of the oyster industry; however, nothing is known about the public health risks related to contamination of these oysters with human pathogens. Commercial size *C. ariakensis* triploids were maintained in large marine tanks with low (8 ppt), medium (12 ppt) and high (20 ppt) salinity water spiked with 1.0×10^5 of transmissive stages of the following human pathogens: *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts and microsporidian spores (i.e., *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Enterocytozoon bieneusi*). Viable oocysts and spores were still detected in oysters on day 33 post water inoculation (pwi), and the cysts on day 14 pwi. The recovery, bioaccumulation, depuration, and inactivation rates of human waterborne pathogens by *C.*

ariakensis triploids were driven by salinity, and were optimal in medium and high salinity water. The concentration of human pathogens from ambient water by *C. ariakensis* and retention of these pathogens without (or with minimal) inactivation and a very slow depuration rate, provides evidence that these oysters may present a public health threat upon entering the human food chain if harvested from polluted water. This conclusion is reinforced by the concentration of waterborne pathogens used in the present study which was representative of levels of infectious agents in surface waters including the Chesapeake Bay. Aquacultures of non-native oysters in the Chesapeake Bay will provide excellent ecological services in regards to efficient cleaning of human infectious agents from the estuarine waters.

OFFSHORE MOLLUSCAN SHELL ACCUMULATIONS: OCEAN RUBBISH OR REFUGE?

Kaitlin Graiff (Jackson Estuarine Laboratory, University of New Hampshire), Ray Grizzle (Jackson Estuarine Laboratory, University of New Hampshire), Holly Abeels (Jackson Estuarine Laboratory, University of New Hampshire), Jennifer Greene (Jackson Estuarine Laboratory, University of New Hampshire), Melissa Brodeur (Jackson Estuarine Laboratory, University of New Hampshire), Larry Ward (Jackson Estuarine Laboratory, University of New Hampshire)

Molluscan shell accumulations were mapped in a 400 km² study area in the western Gulf of Maine off the New Hampshire coast during 2004 using towed underwater video. These shell accumulations were mainly found along the edges of Jeffreys Ledge and were comprised of empty valves of the Atlantic surf clam (*Spisula solidissima*) and the ocean quahog (*Arctica islandica*). Although there has been little ecological research on offshore shell accumulations, they have been identified as potentially significant habitat for several fish species and invertebrates such as lobsters and crabs. Shell accumulations increase the complexity of the sea floor and potentially provide refuge from predators for juvenile life stages of finfish and invertebrates. Subtidal oyster shell (*Crassostrea virginica*) accumulations in southeastern U.S. estuaries are known to provide habitat for many species of commercially important fish and crustaceans. It seems reasonable to expect a similar ecological role for offshore accumulations of shells from other molluscs. Preliminary assessment of the video data from Jeffreys Ledge also indicates a possible relationship between burrowing “anemone forests” (cerianthids) and observed shell accumulations. Future work should explore the habitat value that offshore shell accumulations potentially have for managed species.

AQUACULTURE CAGE BIOFOULING IN THE GULF OF MAINE: HOW DOES THE BLUE MUSSEL (*MYTILUS EDULIS*) OUT-MUSCLE OTHER SPECIES?

Jennifer Greene (Jackson Estuarine Laboratory, University of New Hampshire), Ray Grizzle (Jackson Estuarine Laboratory, University of New Hampshire)

Biofouling on naturally occurring hard substrates in the Gulf of Maine has been well documented, and a variety of biotic interactions as well as physical factors have been found to influence settlement and successional patterns. The present study differed from all previous research in the region by focusing on large, offshore fish cages suspended above the seabed. The overall goal was to characterize development of fouling communities, while providing information for engineers and project managers. Biofouling, especially by heavier organisms like mollusks, adds weight and drag to fish cages, thereby reducing water flow and affecting its behavior in rough seas and high currents. Experimental panels were deployed at a depth of ~15 meters over four different time intervals, in replicates of four, over the course of one year. One set of panels was deployed for a full year, two sets for 6-months (beginning at different seasons), and four sets of 3-month (seasonal) panels. Each panel was constructed of a 10x10 cm piece of knotless nylon netting attached to a PVC frame. The fouling community that developed diverged from the pattern previously described for shallow water benthos, mainly because of the dominance of the blue mussel, *Mytilus edulis*, in nearly all seral stages as well as the climax (1-year) fouling community. Factors probably

influencing this dominance included limited predator abundance, optimal growth conditions, and high reproductive rates.

DEVELOPMENT OF NOVEL TECHNOLOGIES TO COMBAT FOULING IN AQUACULTURE

Paul Gribben (University of New South Wales), Tim Charlton (University of New South Wales), Lachlan Yee (University of New South Wales), Rocky De Nys (James Cook University), Peter Steinberg (University of New South Wales)

Fouling of nets, pens, and buoys, and in some cases (shellfish) the organisms themselves, is a major problem for the aquaculture industry. In Australia, a conservative estimate of the costs of fouling to the industry is \$20-30M, in lost production, cleaning and other labor costs, etc. The use of traditional solutions for fouling control (mostly derived from antifouling technologies for ships) is problematic in aquaculture systems because of environmental, health (proximity to food stuffs) and cost constraints. At the centre for Marine Biofouling and Bio-Innovation (University of New South Wales and the School of Marine Biology and Aquaculture (James Cook University), we have been developing a variety of novel solutions for use in aquaculture. These have developed for a number of industry sectors, and span technologies that are being commercialised to those that are early stage experimental science. Early stage commercialisation technologies are heavy metal free, water based coatings using organic biocides for use on salmon cages and rapid dry coatings for direct application to pearl and edible oysters. New technologies under development include low cost coatings using deterrent surface properties, and living paints encapsulating marine bacteria.

IN SITU MEASUREMENTS OF SESTON UPTAKE BY CONSTRUCTED/RESTORED OYSTER REEFS IN SOUTH CAROLINA

R.E. Grizzle (Jackson Laboratory & Dept. of Zoology, Univ. New Hampshire), J.K. Greene (Dept. of Zoology, University of New Hampshire), L.D. Coen (MRRI, SC Dept. Natural Resources), N. Hadley (MRRI, SC Dept. Natural Resources)

One of the metrics that can be used to assess the success of restored oyster reefs is their effect on water quality. This presentation describes a novel technique we have developed to provide rapid assessment of seston uptake (removal) by dense populations of bivalve molluscs, focusing on data gathered during May 2005 from five shellfish reefs of different ages, bivalve densities, and other characteristics in South Carolina. The overall technique involved concurrent sampling upstream and downstream of each study reef with *in situ* fluorometers and pumped water for laboratory analyses. *In situ* fluorometry detected significant differences between mean upstream and downstream readings for all five reefs, with total seston uptake ranging from 1.9% to 23.1%. Laboratory analysis of chlorophyll *a* concentrations in pumped water samples taken concurrently with the *in situ* fluorometry data showed substantial variability between upstream and downstream data, except for one reef which indicated an overall 35% uptake rate (compared to 23.1% based on *in situ* fluorometry data). As a control, sampling over recently constructed reefs (shell only) did not result in any measurable seston loss from the water column. Our system is a fast and effective approach to quantifying seston removal, and when data are logged at short-term intervals (seconds) potentially provides information on variations in feeding rates and related processes typically only available in laboratory studies.

EARLY SREAMER CLAM (MYA ARENARIA) IN TWO COASTAL PONDS IN OAK BLUFFS, MA (MARTHA'S VINEYARD ISLAND)

David W. Grunden (Shellfish Department Town of Oak Bluffs), Danielle Ewart (Shellfish Department Town of Oak Bluffs)



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The investigation into this project began in 2001 when the Town of Oak Bluffs purchased 200,000 seed (2-3mm) steamer clams.

Three trial restoration sites were chosen; two in Sengekontacket Pond and one in Lagoon Pond. One site in Sengekontacket Pond has remained open for harvest. The other two sites are alternated one opening every other year. All three sites are utilized primarily for recreational shellfishing, though occasionally a commercial shellfisherman does harvest from one of the sites in Sengekontacket Pond.

The first year the steamer seed was only grown in the upweller and broadcast seeded at 12-15mm. The seed at this size dug into the sandy substrate within 15 minutes. The area was marked but ice removed the marker that first winter.

In subsequent years the Town of Oak Bluffs has been able to increase the amount of seed raised to 600,000 in 2005 (another 600,000 has been ordered for the 2006 season). The Town's Shellfish Department constructed sand filled nursery rafts to allow for greater growout of the seed. The seed steamers are still removed from the upweller once they reach 12-15mm, but are now transferred into the nursery rafts where they are held until late mid-September or early October. The seed measured 22.4 – 38.2 mm when they were planted out in the fall of 2005.

The fishing effort has been monitored by the Shellfish Department and we have seen a modest increase in the steamer clam landings from these areas since the project began.

OYSTERS AND OYSTER FARMING IN CHINA: A REVIEW

Ximing Guo (Haskin Shellfish Research Laboratory, Rutgers University), Guofan Zhang (Institute of Oceanology, Chinese Academy of Sciences, PRC), Lumin Qian (Third Institute of Oceanology, Oceanic Administration, PRC), Haiyan Wang (Haskin Shellfish Research Laboratory, Rutgers University), Xiao Liu (Institute of Oceanology, Chinese Academy of Sciences, PRC), Aimin Wang (Ocean College, Hainan University, PRC)

In an effort to survey *Crassostrea ariakensis* populations in China, we conducted literature reviews and visited over 37 sites along China's coast. Here we present our findings about oysters and oyster farming in China in light of recent taxonomic revisions. Seventeen species of oysters have been reported along China's coast. Many of the species occur in southern China and are relatively rare. There is considerable confusion about the classification of *C. ariakensis* and three other species. Numerous oyster reefs, both ancient and living, exist along the coast, and *C. ariakensis* was present in all four live reefs that we saw. In three of the reefs, *C. ariakensis* is the dominant or founding species, where large oysters on the bottom are *C. ariakensis* and small oysters on top are other species. Oyster farming is primarily for *C. hongkongensis* in the south (Guangxi and Guangdong), *C. gigas* in the north (Shandong and Liaoning), and *C. angulata* in the middle (Fujian and Zhejiang). *C. hongkongensis*, also known as the white oyster or *C. rivularis*, is one of the most important species cultured in China. *C. hongkongensis* culture is based on natural seeds with one estuary in Guangxi supplying about 2.5 billion. Most of the published literature on *C. rivularis* from southern China is for *C. hongkongensis*, not for *C. ariakensis*, which is not intentionally cultured. It is present at low frequencies in natural *C. hongkongensis* seeds and selected against by farmers. Pollution has devastated oyster populations in at least two estuaries.

GENOME MAPPING IN THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA* GMELIN)

Ximing Guo (Haskin Shellfish Research Laboratory, Rutgers University), Yongping Wang (Haskin Shellfish Research Laboratory, Rutgers University), Ziniu Yu (Haskin Shellfish Research Laboratory, Rutgers University), Lingling Wang (Haskin Shellfish Research Laboratory, Rutgers University), Jeong-ho Lee (Haskin Shellfish Research Laboratory, Rutgers University)

The eastern oyster, *Crassostrea virginica* Gmelin, is an important aquaculture species as well as a popular model species for molluscan research. The biology and ecology of the eastern oyster are well understood, but our

knowledge about its genome remains limited. We have been developing tools and applying them in genome mapping in the eastern oyster. For physical mapping, we used fluorescence in situ hybridization (FISH) to characterize and map oyster chromosomes. Repetitive DNA sequences and genes were mapped to oyster chromosomes by FISH, and some revealed interesting features of the oyster genome. The chromosomal location of rRNA genes, on the long arms of Chromosome 10 in Pacific species and on the short arms of Chromosome 2 in Atlantic species, provided an interesting divide between the Pacific and Atlantic species. Nine P1 clones were mapped to seven of the ten oyster chromosomes. For linkage mapping, we developed over 600 amplified fragment length polymorphism (AFLP) markers and constructed four moderately dense linkage maps. We identified disease-resistance QTL by mapping markers that showed significant frequency shifts after disease-inflicted mortalities. Markers affected by diseases clustered together on genetic maps, and all affected markers within a cluster showed frequency shifts in the same direction. Mapping analysis in two families identified 12 putative Dermo/summer mortality-resistance QTL, and at least seven were independent. Microsatellites are being developed and mapped to increase the transferability of genetic maps and mapped QTL. Candidate host-defense genes are being mapped using single nucleotide polymorphism to detect possible linkage to disease-resistance QTL.

TESTING DISEASE RESISTANCE IN OYSTERS: EXPERIMENTAL INFECTIONS WITH BACTERIAL PATHOGENS IN CRASSOSTREA VIRGINICA LARVAE AND SPAT

Javier Gómez-león (University of Rhode Island), Rachel Hadley (University of Rhode Island), Luisa Villamil (University of Rhode Island), Marta Gómez-chiari (University of Rhode Island)

Culture of the American oyster (*Crassostrea virginica*) is a traditional activity that has great economical importance in the East Coast of USA and the Gulf of Mexico. Globally, shellfish production is often affected by bacterial pathogens, mainly *Vibrios*, which lead to high mortality rates in shellfish hatcheries. Another bacterial disease that has heavily impacted oyster culture in the Northeast US is Juvenile Oyster Disease (JOD), thought to be caused by *Roseovarius crassostreae*. In the present study, bacterial isolates that caused important mortalities in Pacific oyster larvae, RE22 and RE101, as well as an isolate from JOD-affected oysters (CV919-312) were used to perform experimental infections of oyster larvae and spat in order to determine differences in the susceptibility to bacterial infection of three oyster lines: a local Rhode Island line, a line resistant to Dermo and MSX (NEH), and a line resistant to JOD (FMF). All bacterial isolates tested were able to induce significant mortality in larvae and spat of *C. virginica*, reaching mortalities ranging from 50 – 100%. Differences in susceptibility between the lines were observed, with the NEH line showing the highest survival. Infected larvae exhibited abnormal circular swimming movements on their sides and deformed velum with cilia clumping. Factors affecting survival included temperature and size of the oysters. This research may provide a useful tool to test for disease resistance mechanisms in oysters.

EFFECT OF DIETARY PROTEIN ON CONSUMPTION, GROWTH AND PRODUCTION OF THE SEA URCHIN LYTECHINUS VARIEGATUS

H. Hammer (Dept of Biology, University of Alabama at Birmingham), *s. Watts (Dept of Biology, University of Alabama at Birmingham), A. Lawrence (Shrimp Mariculture Project Texas A&M University), J. Lawrence (Dept of Biology, University of South Florida)

Feeds that differ in protein concentration (17, 21, 25 and 31%, as fed) were formulated, cold-extruded and dried at 60C to produce a dry pellet. Sea urchins (n=64) were divided into 4 groups and placed individually into a semi-recirculating system at 32 ± 2‰ salinity and 22 ± 1C. Urchins were fed daily 1 of 4 diets *ad libitum* for 87 days. Consumption was measured daily; urchins were weighed and test diameters measured at days 0, 29, 57 and 87. At day 87, urchins were dissected and separated into components (test, lantern, gut and gonad) that were weighed, dried and reweighed. At days 0- 87, urchins fed the 31% protein feed consumed less food than the other treatments. Urchins fed the 31% protein feed had a significantly greater body weight gain/day (1.3%) and test diameter (42.7mm) than urchins fed the 17% (0.9%, 38.3mm, respectively) or 21% protein feed (1.1%, 40.7mm, respectively) but not the 25% protein feed (1.3%, 41.7mm, respectively). Wet weight was significantly different among the treatments and varied directly with dietary protein (40.4, 37.0, 34.0, 28.6g, respectively). Urchins fed the 31% protein feed had the highest production efficiency (48.1%) among the treatments (36.6, 32.5 and 24.8%, respectively). Gonad production efficiency in urchins fed the 31% protein feed (12.6%) was greater than urchins fed



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the 17% (6.9%) or 21% (9.4%) but not the 25% protein feed (10.7%). The 31% protein feed provided the best weight gain with the highest efficiency of the feeds tested. Supported by Mississippi-Alabama SeaGrant Consortium.

DEMOGRAPHIC CHARACTERISTICS OF STABLE FRESHWATER MUSSEL POPULATIONS IN THE SOUTHEASTERN UNITED STATES

Wendell R. Haag (USDA Forest Service Center for Bottomland Hardwoods Research), Melvin L. Warren Jr. (USDA Forest Service Center for Bottomland Hardwoods Research)

Population dynamics of freshwater mussels are poorly known and represent a major information need for conservation of these imperiled animals. Most notably, the level of recruitment needed to maintain a stable mussel population is unknown, making it difficult to meaningfully assess the viability of a population. We measured annual recruitment and survivorship in two diverse mussel communities (Little Tallahatchie River, MS, and Sipsey River, AL) from 1999 to 2005. For all species, survivorship from glochidia to the recruit stage (benthic individuals approximately 2-4 months of age) was low (9.12×10^{-6} to 3.92×10^{-5}), but was high for all other life stages (0.63-0.95). Recruitment varied widely among species, sites, and years. For some species (e.g., *Amblema plicata*, *Elliptio arca*, and *Obovaria unicolor*), recruitment was sporadic, ranging from years with no detectable recruitment to years in which recruits composed > 50% of the population. For other species (e.g., *Fusconaia cerina*, *Pleurobema decisum*, *Quadrula asperata*, and *Q. pustulosa*), recruitment occurred at a more constant level (0-15% of population). We used stochastic stage-based matrix population models based on observed demographic parameters to evaluate the potential influence of varying levels of recruitment on long-term population viability. These models predict that species with higher annual variability in recruitment require a higher mean recruitment level over time to maintain a stable population than species with less variable recruitment. Demographic differences among species show the existence of widely divergent life history strategies in freshwater mussels and have important implications for the conservation of these animals.

IMPACT OF SEA OTTERS ON SHELLFISH FISHERIES AND AQUACULTURE IN B.C. CANADA

Rick Harbo (Fisheries and Oceans Canada), Linda Nichol (Fisheries and Oceans Canada), Laurie Convey (Fisheries and Oceans Canada), Jennifer Toole (Fisheries and Oceans Canada), Lieneke Marshall (Fisheries and Oceans Canada)

Re-introduced sea otters have increased in number (>3200 estimated in 2005) and expanded their range on the British Columbia coast, causing impacts on shellfish fishery resources. There have been concerns expressed by First Nations about the loss of access to clam, crab and sea urchin resources. The increase in kelp beds (a result of otter predation on urchins) has affected local navigation channels for small boats.

A variety of commercial and recreational shellfish fisheries have been impacted by sea otters, including Dungeness crab, *Cancer magister*, red sea urchin, *Strongylocentrotus franciscanus*, Geoduck clam, *Panopea abrupta*, and Manila clam, *Venerupis philippinarum*. While otters are not the only cause of shellfish declines off BC (e.g., northern abalone, *Haliotis kamtschatkana*), predation by otters will keep shellfish abundances and sizes at levels that hinder the resumption of commercial fisheries.

Sea otters have limited plans for geoduck aquaculture and enhancement on the west coast of Vancouver Island. There have been complaints about otter predation on some intertidal manila clam aquaculture tenures. To date, sea otters have not taken an interest in Pacific oysters on intertidal or deep water aquaculture tenures. It is expected that conflicts will continue and increase in the next 10 years.



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AGE AND GROWTH OF WILD *CRASSOSTREA ARIAKENSIS* AND *C. GIGAS* FROM LAIZHOU BAY, CHINA

Juliana M. Harding (VIMS, Gloucester Point, Virginia, USA 23062), Roger Mann (VIMS, Gloucester Point, Virginia, USA 23062)

Shell height at age estimates from Suminoe (*Crassostrea ariakensis*) and Pacific (*Crassostrea gigas*) oysters from a natural oyster reef in Laizhou Bay, China were compared with estimates from triploid *C. ariakensis* of known age from the Rappahannock River, Virginia. Both *C. ariakensis* and *C. gigas* reach shell heights in excess of 76 mm (3 inches) within two years after settlement regardless of the source location. This fast growth appears to continue through at least Age 4 or Age 5 in wild individuals as the growth trajectory for both species had not begun to flatten in the oldest individuals collected. Fitted growth curves were not significantly different between species within the same habitat, within species in different habitats or between species in different habitats.

TROPHIC CONSEQUENCES OF A LONG LIVED NON-NATIVE PREDATOR *RAPANA VENOSA* ON ESTUARINE COMMUNITY DYNAMICS

Juliana M. Harding (VIMS, Gloucester Point, Virginia, USA 23062), Roger Mann (VIMS, Gloucester Point, Virginia, USA 23062)

Veined rapa whelks *Rapana venosa* are large generalist predators with the potential to live in excess of 10 yrs and life history suitable for successful invasion of estuarine habitats. The presence of rapa whelks in a habitat has obvious consequences for the prey field in that rapa whelk prey consumption shifts ontogenetically from small (e.g., *Mytilus* sp., *Macoma* sp., *Mya* sp., *Crassostrea* sp.) to large (e.g. *Mercenaria* sp, *Crassostrea* sp.) prey. Laboratory and mesocosm experiments indicate that wild rapa whelks reach a size refuge from predation by blue crabs (*Callinectes sapidus*) within one year post settlement at critical sizes of 30-40 mm SL. At sizes >40 mm, rapa whelk distribution in Chesapeake Bay is probably limited only by salinity and food availability. The presence of large predators with the ability to repel competitors (blue crabs) on shared feeding grounds may force native species into habitat refugia delimited by salinity tolerances. Scenarios predicting relative abundance and competitive interactions between bivalve prey, rapa whelks, and blue crabs with impacts on trophic dynamics and habitat use are discussed.

GENETIC TRACKING OF RESTORATION OYSTERS TO GAUGE SUCCESS – A COST/BENEFIT ANALYSIS

Matthew P. Hare (University of Maryland)

Restoration of oyster stocks in Chesapeake Bay is a monumental task to which abundant resources have been committed and for which many dedicated parties have labored. Beyond establishing a ten-year goal for the increase of census numbers, little attention was initially paid to establishing rigorous benchmarks for success, or collecting data that could either measure small successes or determine the cause of failures. This has been changing more recently, but now most restoration effort is focused on targeted population supplementation using artificially selected, disease tolerant *C. virginica* to combat high mortalities from parasitic diseases. This tactic has potential benefits and considerable risks. I will argue that genetic testing of recruitment is necessary to evaluate and manage the genetic risks of supplementation with inbred oysters, but it also provides the most meaningful measure of overall restoration efficacy. Results will be presented from a highly collaborative effort to genetically monitor DEBY-strain restoration plantings in two Chesapeake subestuaries. The results indicate that the hatchery amplification of DEBY broodstock to produce restoration oysters is contributing to inbreeding in the oysters over and above that already realized during artificial selection. Also, the DEBY contribution to local recruitment in the Great Wicomico River in 2002 was approximately 10%, a result that belies appearances based on the number of DEBY oysters planted and

the elevated levels of recruitment that year. Inbreeding may or may not be the greatest risk facing restoration oysters and threatening restoration success, but only improved genetic testing will allow informed risk management.

METABOLIC RATES OF *CRASSOSTREA ARIAKENSIS* AND *CRASSOSTREA VIRGINICA* AT TWO TEMPERATURES AND THREE SALINITIES

Nicole Harlan (University of Maryland), Kennedy Paynter (University of Maryland), Donald Meritt (University of Maryland Center for Environmental Science)

Maryland and Virginia have proposed to replace the native oyster, *Crassostrea virginica*, with the suminoe oyster, *Crassostrea ariakensis*, in Chesapeake Bay. *C. virginica*, is highly tolerant of hypoxic conditions and can survive emersion or nearly anoxic seawater for days to weeks depending on the temperature. In order to replace *C. virginica*'s ecological niche of establishing vast benthic reefs in Chesapeake Bay, *C. ariakensis* may require similar tolerances. However, when the oysters were placed in sealed jars of anoxic water, *C. ariakensis* lived for an average of four days, while *C. virginica* persisted for more than fourteen days. Studies at 22°C have shown that the metabolic rate of *C. ariakensis* (1.96 +/- 0.102 O₂/hr/gdw) is significantly higher than that of *C. virginica* (1.15 +/- 0.079 mg O₂/hr/gdw; p=0.0244). In order to better understand the aerobic requirements of these two species under different conditions, standard metabolic rates of each species were determined at two temperatures, 10 and 20°C, and three salinities, 5, 15, and 25 psu. At both temperatures and all three salinities, the metabolic rate of *C. ariakensis* was higher than that of *C. virginica*. Upon immersion in the test chambers, *C. ariakensis* gaped and began using oxygen within minutes, while *C. virginica* kept their valves shut for much longer. These data may influence the decision to use *C. ariakensis* as an ecological substitute for *C. virginica* in the Chesapeake Bay.

DEVELOPMENT OF PCR TECHNIQUES FOR THE DETECTION OF *VIBRIO CARCHARIAE* AND *PERKINSUS OLSENI* IN ABALONE PARAFFIN EMBEDDED TISSUES.

Leanne Harris (Dublin Institute of Technology), Fergus Ryan (Dublin Institute of Technology), Helen Lambkin (Dublin Institute of Technology), Nuala O'Byrne-ring (Dublin Institute of Technology)

Global demand for abalone has significantly increased in recent years, however wild stocks of abalone have declined in number, resulting from overexploitation as well as the spread of infectious disease. Disease outbreaks are recognised as a significant constraint to aquaculture production and trade that affects both economic development and the socio-economic revenue of many countries.

Bacteria and protozoa are the most commonly encountered pathogens in abalone shellfish. *Vibrio harveyi/carchariae* is a highly pathogenic bacterium to abalone and *Perkinsus atlanticus/olseni* is a protozoan that also causes severe disease in this shellfish. Both pathogens are ranked amongst the top ten most significant disease causing organisms of abalone.

In this study a multiplex PCR method was developed to simultaneously amplify a 413 bp region of the 16S rRNA sequence of *Vibrio carchariae/harveyi* and a 155 bp region of the actin mRNA gene sequence of *Haliotis* spp. This multiplex PCR was used to amplify these sequences in both fixed tissues and paraffin embedded tissues of infected *Haliotis tuberculata*.

A primer set was designed to target a 245 bp region of the ITS sequence of *Perkinsus atlanticus* from paraffin embedded samples of infected *Ruditapes decussatus* which could be adapted to detect *Perkinsus olseni* in paraffin embedded abalone tissues. Also quantitative PCR using these primers is a further potential development.

These PCR protocols offer a rapid and specific method for the identification of *Vibrio carchariae* and *Perkinsus olseni* in shellfish.

ANALYSIS OF ENZYME, CARBOHYDRATE AND MINERAL DISTRIBUTION IN THE FOOT OF ABALONE SHELLFISH.

Leanne Harris (Dublin Institute of Technology), Helen Lambkin (Dublin Institute of Technology), Nuala O'Byrne-ring (Dublin Institute of Technology)

The meat of the abalone shellfish has been labelled as a luxury food for thousands of years. The source of this epicurean delicacy is the foot, which is the most conspicuous external feature of this organism. The foot is a large

muscular organ with an extensive nerve and vascular supply that serves both sensory and locomotory functions. The foot is also involved in many other functions such as locating and manipulating food, attaching eggs to substrates, cleaning the shell, finding potential mates and thwarting predators. The foot is primarily made up of epithelial tissue, connective tissue and muscle.

In this study the distribution of functional and structural elements was investigated in the pedal organ of two species of abalone, *Haliotis tuberculata* and *Haliotis discus hannai*. The pedal and peripheral epithelia expressed high levels of activity for the following enzymes: chloroacetate esterase, a-naphthyl butyrate esterase, alkaline and acid phosphatase, peroxidase and carbonic anhydrase. The sub-epithelial ganglion cells were positive for a-naphthyl butyrate esterase and acetylcholinesterase. Neutral mucins, acid mucins, carboxylated mucins and sulphated mucins were found in epithelial cells, in sub-epidermal gland cells and in the ground substance of the connective tissue and muscle. Melanin was identified in the sub-epidermal gland cells and in the pedal and peripheral epithelial cells. The basement membrane of the pedal epithelium was positive for calcium.

A myriad of cell components and cellular activities in the tissues of the abalone foot were demonstrated, revealing cell types and reflecting the molecular pathways at work within these tissues.

COMPARATIVE GROWTH AND SURVIVAL OF DIPLOID AND TRIPLOID SUMINOE OYSTERS, *CRASSOSTREA ARIAKENSIS*, IN MULTIPLE QUARANTINE SYSTEMS

Heather D. Harwell (Virginia Institute of Marine Science), Standish K. Allen Jr. (Virginia Institute of Marine Science)

Much of the research on *Crassostrea ariakensis* has revealed superior growth rates and resistance to disease compared to the native oyster, *C. virginica*. All field studies of growth and survival have utilized sterile triploid oysters for reasons of biosecurity. Thus, triploids are serving as a surrogate for diploid performance in these field trials. A direct, simultaneous comparison of the growth and survival of diploid and triploid *C. ariakensis* is needed to refine population growth models based on triploid field data. Three replicate lines of diploid and triploid *C. ariakensis* were placed at four quarantine systems in Virginia and Maryland in December 2004. Individual repeated measures of subsets of oysters were gathered from monthly data on percent survival, wet weight, and shell length. In addition, quarterly estimates of condition index were obtained. Data gathered in this fashion will be used to determine a correction factor(s) that can then be applied to results from past studies of triploid *C. ariakensis* in order to refine models of potential population growth.

ROLE OF LOW SALINITY REFUGE IN REGULATING THE PREVALENCE OF THE PARASITE *LOXOTHYLACUS PANOPAEI* IN THE XANTHID *PANOPEUS OBESUS*

Lesli Haynes (Florida Gulf Coast University), S. Gregory Tolley (Florida Gulf Coast University), Aswani K. Volety (Florida Gulf Coast University), James T. Winstead (United States Environmental Protection Agency)

ABSTRACT: This study was conducted to examine the potential influence of salinity, a proxy for freshwater inflow, on the prevalence of the castrator parasite *Loxothylacus panopaei* on mud crabs found on Southwest Florida oyster reefs. Spatial and seasonal patterns of the presence of potential host crabs and the prevalence of the parasite were assessed in the Caloosahatchee, Estero, and Faka-Union estuaries of Southwest Florida. Lift nets (1 m²) containing 5 liters of oyster clusters were deployed on intertidal reefs at three sites along the salinity gradient of each estuary. Nets were deployed during three seasonally dry and three seasonally wet months for a period of 30 d. Although *Panopeus obesus* were collected at all three locations within each estuary, densities tended to increase downstream in higher salinity waters. Parasite prevalence decreased at the upper stations in each estuary, was reduced during wet months compared to dry months, and was lower for those estuaries that experienced high levels of freshwater inflow. Furthermore, parasite prevalence was positively correlated with the mean salinity at capture of host crabs. Based on the distribution of *P. obesus* and the above patterns related to salinity, it appears that freshwater inflow and seasonal rains might regulate the prevalence of this castrator parasite in Southwest Florida by creating spatiotemporal, low salinity refuge for its host.



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MAPPING GENES AFFECTING SHELL COLOR AND SHAPE IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS*

Dennis Hedgecock (University of Southern California), Philip Grupe (St. Olaf College), Marie-luise Voigt (Institut für Pflanzengenetik und Kulturpflanzenforschung)

Variation in the color and shape of oyster shells is widely believed to be environmentally determined. However, Brake et al (2004 *Aquaculture* 229:89) recently provided evidence that shell and mantle edge color in the Pacific oyster *Crassostrea gigas* are heritable. We observed variation in shells of an F₂ family of Pacific oysters that appeared to be inherited, and we tested this hypothesis by statistical analyses and genetic mapping. Measures of shell shape, size and color were made from digital photographs of 179 oysters, using ImageJ software. Principal component analyses were used to consolidate the measured traits, and mapping was done on both principal components and some of the original individual traits. Mapping was made possible by a previously compiled linkage map for this same family of oysters, comprising 59 microsatellite DNA markers on 11 linkage groups. Using an interval mapping method, we identified quantitative trait loci (QTL) for pigment saturation of the left valve and for a left bend in anteriorly viewed valves ("hook hinge"?). Both QTL were located on linkage group IX (*ucdCgi171*, *ucdCgi189*, *ucdCgi140*, *ucdCgi115* and *uscCgi206*), and explained 13% of the variance in valve bending and 16% of the variance in saturation of left valve pigments. Both traits appeared to be recessive. These observations suggest that genes, as well as environment play, a role in determining the color and shape of oyster shells. Markers associated with these QTL could be used to breed oysters with more desirable shell characteristics.

MANGROVE OYSTERS AS BIOINDICATORS IN SUB-TROPICAL DRY CLIMATES

Heidi Hertler Ph.D. (Interamerican University of Puerto Rico), Lindsay Martinez (Interamerican University of Puerto Rico), Danielle Kreeger Ph.D. (Partnership for the Delaware Estuary), Graciela Ramirez-toro Ph.D. (Interamerican University of Puerto Rico)

Marine systems of the Caribbean are increasingly impacted by changes in land use. Escalating development is in many places out-pacing the ability of existing upland, salt flats, and mangroves to intercept sediment and nutrient runoff flowing toward the adjacent marine system. Elevated concentrations of chlorophyll-a and total suspended solids were correlated with land development in Southwest Puerto Rico. However, a direct cause and effect relationship has not yet been established between development on the land and water quality degradation in the adjacent marine community. Suspension-feeding bivalves are regarded as excellent bioindicators of water quality, but they have not been studied in this context in tropical waters. We examined the population size structure and physiological condition of mangrove oysters, *Crassostrea rhizophorea*, along a gradient of land development in Southwest Puerto Rico in a first step towards using this species as a bioindicator of water quality in sub-tropical environments. Data suggest that *C. rhizophorea* fares better in undisturbed, clearer water rather than in eutrophied areas near development where food availability may be greater. In addition, oyster health, as measured by condition index, was inversely correlated with increasing land use. Thus, physiological measures of *C. rhizophorea* fitness may be useful indicators of ecosystem integrity.

DEVELOPMENT AND APPLICATION OF MICROSATELLITE MARKERS TO DETERMINE THE IMPACT OF RESTORATION OF THE BAY SCALLOP

Elizabeth Hemond (University of North Carolina Wilmington), Ami E. Wilbur (University of North Carolina Wilmington)

A number of marine species, including the bay scallop (*Argopecten irradians*), are being restored using aquaculture-propagated broodstocks. Because these projects are laborious and expensive, it is important to be able to quantify the impact of these restorations. Natural genetic variation within species can be used to distinguish between the progeny of native populations and restoration stocks and thereby quantify the relative impact, provided sufficient genetic markers are available, and that the native and restoration stocks are differentiated to some degree. To facilitate these efforts, we have developed a set of microsatellite loci for bay scallops. Microsatellites are highly polymorphic, tandemly repeated sequences of 2-6 nucleotides dispersed throughout the genome of most eukaryotic organisms. Due to their number and variability, microsatellites are a powerful tool for identifying differences among populations (even individuals) and when used in conjunction with multi-locus analytical methods can

provide the requisite discrimination needed to quantify the impact of restoration. We have isolated 19 trinucleotide and 41 tetranucleotide microsatellite loci and are in the process of characterizing the markers with respect to the level of polymorphism exhibited, presence of null alleles, and fidelity of inheritance. Preliminary data on four loci (AICL112, AICL115, AICL131, and AICL271) revealed extensive polymorphism (37, 14, 17 and 10 alleles, respectively) in two populations from Florida (Anclote Estuary and Pine Island Sound), but surprisingly little differentiation between locations (Monte Carlo X^2 , allele frequency distribution, $p > 0.05$). Results from additional loci will be presented and the utility of these markers in assessing restoration will be discussed.

CAN HARD CLAM LARVAL SURVIVAL EXPLAIN RECRUITMENT FAILURE IN GREAT SOUTH BAY: A MODELING STUDY?

Eileen E. Hofmann (Old Dominion University), Eric N. Powell (Haskin Shellfish Research Laboratory, Rutgers University), John M. Klinck (Old Dominion University), John N. Kraeuter (Haskin Shellfish Research Laboratory, Rutgers University), Rebecca Marzec (Haskin Shellfish Research Laboratory, Rutgers University), V. Monica Bricelj (Canada Natural Resources Council)

A biochemically-based model was developed to simulate the growth, development, and metamorphosis of hard clam (*Mercenaria mercenaria*) larvae. Larvae are simulated in terms of protein, neutral lipid, polar lipid, carbohydrate, and ash content. Initial biochemical content of the larva is determined by egg composition and changes in this occur as the larva grows and in response to the biochemical composition of available food. Simulations that used environmental conditions from Great South Bay, Long Island showed that variations in temperature and food quantity produce small changes in overall larval survivorship relative to that obtained from average conditions. The largest decrease in larval survivorship resulted from variations in the quality of food available to the larva. Reductions in food lipid content resulted in lowest larval survival rate. These results suggest that changes in food quality during the past decade may be a contributing factor to the present low recruitment rates of hard clam larvae in Great South Bay.

LINKING MARINE PATHOGENS TO MOLLUSCAN SHELLFISH: THE ECOLOGICAL ROLE OF MARINE AGGREGATES

Bridget A. Holohan (University of Connecticut), M. Maille Lyons (University of Connecticut), J. Evan Ward (University of Connecticut), Roxanna M. Smolowitz (Marine Biological Laboratory), Kevin R. Uhlinger (Marine Biological Laboratory), Joseph J. Vallino (Marine Biological Laboratory), Bassem Allam (Stony Brook University)

Benthic suspension-feeding invertebrates, such as bivalve molluscs, are constantly exposed to settling aggregates and the variety of microorganisms they contain. Recent research in our laboratory demonstrates that aggregates can be a source of food particles as well as a reservoir for disease organisms. The field portion of this project is part of a large collaborative study that is addressing the ecological role of marine aggregates as a link between oysters (*Crassostrea virginica*) and the pathogen *Perkinsus marinus* and between northern quahogs (*Mercenaria mercenaria*) and the pathogen Quahog Parasite Unknown (QPX). Reference sites with low or no mortalities from these pathogens are located in Connecticut. Disease sites are located in Massachusetts and New York. At all sites, aggregate samples are collected using settling cones for the determination of the presence and concentration of pathogens. Concurrently, suspended aggregates are recorded *in situ* using a video camera focused on a stationary grid that is mounted within centimeters of the camera lens. The size and number of aggregates are determined using an image analysis program. Temperature, salinity, water depth and tidal stage are also recorded to evaluate correlations among aggregate characteristics (number and size), environmental parameters and pathogen presence. A summary of year one samples, taken from April through November, 2005 will be presented.

AUTOGENIC ECOSYSTEM ENGINEERS AND THE INFLUENCE OF HABITAT COMPLEXITY ON INTERTIDAL MIGRATIONS BY A TRANSIENT PREDATOR.

Kirstin K. Holsman (School of Aquatic and Fishery Sciences, UW), P. Sean McDonald (School of Aquatic and Fishery Sciences, UW), David A. Armstrong (School of Aquatic and Fishery Sciences, UW)



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Autogenic ecosystem engineers, which physically alter the systems they inhabit, have cascading impacts on various organisms. There are numerous examples of elevated densities within complex habitats created by native, naturalized, and exotic engineering species, yet the facilitative effects of these engineers depends on landscape patterns of their distribution as well as the size of the associated biota. Positive effects of increased physical structure often benefit small resident invertebrates while inhibiting larger transitory species. In this study we examined how physical structure along a gradient of habitat complexity influences patterns of migration and habitat use by a transient benthic predator, Dungeness crab (*Cancer magister*). In particular we compared crab habitat use of unstructured littoral habitats (ULH), native eelgrass beds (*Zostera marina*), naturalized Pacific oyster beds (*Crassostera gigas*), and invasive *Spartina alterniflora* patches in Willapa Bay, WA. Baited trap surveys on ULH yielded catches of subadult *C. magister* 30 - 95% higher than catches from eelgrass beds, oyster beds, and meadows of *S. alterniflora*. Ultrasonic telemetry observations suggest that subadult *C. magister* making nighttime foraging incursions prefer ULH to other littoral habitats, and underwater video observations show that migrations are influenced by tidal rhythms since movements are correlated with the direction and velocity of current flow. The architecture of complex habitats negatively influences *C. magister* utilization of littoral habitats, and ULH may be the primary foraging areas for migrating crabs. Since daily intertidal forays largely subsidize subtidal crab populations, ULH are particularly critical to crab production in these systems.

ARE TRIPLOID HARD CLAMS RESISTANT TO FLORIDA SUMMER STRESSORS? RESULTS OF LABORATORY CHALLENGES.

Elise Hoover (University of Florida, Fisheries and Aquatic Sciences), Shirley Baker (University of Florida, Fisheries and Aquatic Sciences), John Scarpa (Harbor Branch Oceanographic Institution), Leslie Sturmer (University of Florida, Cooperative Extension)

The hard clam, *Mercenaria mercenaria*, is an important aquaculture species in the state of Florida with \$12.9 million reported sales in 2003. Recently, clam farmers in southwest Florida have experienced high clam mortalities during the summer months. Clams may be experiencing heightened physiological stress due to an initial reduction in biomass from a spring spawning event, followed by increasing temperatures, fluctuating salinities and low dissolved oxygen levels. Triploid clams offer a potential solution to this problem as they are functionally sterile and therefore should have more energy available to survive these summer stressors.

Triploidy was induced in the hard clam, *Mercenaria mercenaria*, by suppressing polar body I or II formation in fertilized eggs with cytochalasin B. Survival of triploid and diploid clams will be compared in laboratory challenges as part of a larger project to determine if there is a potential use for triploids in aquaculture. Laboratory challenges will combine typical southwest Florida conditions in a 3 x 2 factorial design. Water temperatures will be held at 32°C (90°F) in all treatments. Clams will be challenged with salinities of 15ppt, 25ppt, and 40ppt and dissolved oxygen will be maintained at either normoxic or hypoxic levels. These lab-based challenge experiments will aid in determining if triploids are more stress resistant compared to diploids, thus increasing survival. Supported by USDA-ARS and FL Sea Grant.

JUVENILE CHINOOK SALMON ONCORHYNCHUS TSHAWYTSCHA UTILIZATION OF LOW-INTERTIDAL EELGRASS AND OYSTER AQUACULTURE BEDS.

Geoff Hosack (Oregon State University), Brett Dumbauld (USDA-ARS), Ian Fleming (Memorial University of Newfoundland)

Estuaries function as nursery habitats for Pacific salmon populations by providing juvenile outmigrating salmonids with refugia from predators and trophic resources. Intertidal habitat in estuaries along the West Coast of the U.S. is often modified by the addition of live oysters in aquaculture operations which have become one of the most significant contributors to small coastal economies. To assess the importance of low-intertidal habitats for juvenile

Chinook salmon, we compared the diet and distribution of Chinook in on-ground oyster aquaculture beds (*Crassostrea gigas*) with that in seagrass *Zostera marina* ("eelgrass") and unvegetated mudflat. We used a modified tow net to compare densities and collect diets of juvenile Chinook salmon (65-115 mm FL) in Willapa Bay, Washington. Using laboratory mesocosms, we evaluated which of these intertidal habitats are preferred by juvenile Chinook as refugia from predators. Field results suggested that outmigrating smolts did not exhibit a strong habitat preference since diet composition was unrelated to habitat type at the point of capture, and densities were significantly related to broad spatial patterns in the estuary but not habitat type within the low-intertidal zone. Older Chinook smolts (81-115 mm FL) in summer laboratory experiments significantly preferred eelgrass to mudflat or oyster in the presence of a mock avian predator while younger Chinook in spring (42-80 mm FL) demonstrated no significant change in habitat preference. This suggests that Chinook may not always exhibit strong habitat specificity, but structured intertidal habitats may provide important resources such as movement corridors on a broad landscape scale.

STATUS, TRENDS AND RESTORATION PLANNING FOR FRESHWATER MUSSELS OF THE COLUMBIA BASIN/PACIFIC SLOPE: THE CTUIR MUSSEL PROJECT

Jeanette Howard (Confederated Tribes of the Umatilla Indian Reservation), Jayne Brim Box (Confederated Tribes of the Umatilla Indian Reservation), David Wolf (Confederated Tribes of the Umatilla Indian Reservation)

The Freshwater Mussel Project of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR), the first program of its kind in the West, explores new ground in the restoration of freshwater mussels. The project began in 2003, and expands on the Tribe's overall goal of recovering an intact, fully functioning, Umatilla River. Freshwater mussels were vital components of stream ecosystems in the Columbia River Basin that have been affected directly and indirectly by dams, habitat deterioration, and declines in salmon populations. Mussels were also culturally and traditionally important to Native Americans in the region. Yet, prior to this study, little was known about the distribution or status of mussel populations in the region. The overall goal of CTUIR's mussel project is to provide essential information for designing a recovery plan in the Umatilla River and other Columbia subbasins where mussels may be declining or extinct. However, this cannot be accomplished without first better understanding habitat and host fish requirements, genetic diversity, functional role, population structure and historic distribution. In this presentation, I will summarize current efforts of the CTUIR Freshwater Mussel Project to fill knowledge gaps, including: the historic and current status and distribution of freshwater mussels in CTUIR's rivers of interest; habitat variables controlling distribution; preliminary information on the functional role of freshwater mussels in these systems; host fishes and timing of reproduction. In addition, I will discuss current efforts underway to develop an ecologically based biomonitoring program using mussels as sentinel bioindicators in the Columbia Basin.

OREGON'S CLATSOP BEACH RAZOR CLAM FISHERIES; PAST, CURRENT AND THE FUTURE Matthew Hunter (Oregon Department of Fish and Wildlife)

In Oregon, the 18-mile stretch of shoreline, known as the Clatsop Beaches, extends from the South Jetty of the Columbia River south to Tillamook Head. Over 90% of Oregon's razor clam catch and effort occurs in this area. This area has had a fishery since the turn of the century and it is where the majority of biological information, recreational and commercial fishery data has been collected. Historically, the fishery has been sampled on low-tide series during the spring and summer months and as weather permitted the rest of the year. Recreational digging effort from 1995-2004 averaged 62,700 digger trips per year with a record high in 2004 of 157,000. Commercial participation from 1995-2004 averaged 125 individuals with a high of 255 individuals in 2002. The total annual harvest from 1995-2004 averaged 896,000 clams with a record harvest in 2002 of 2,660,000 clams. The Pacific razor clam is considered to be the finest food clam on the west coast resulting in the harvest being a popular tradition among coastal and inland residents. Due to this popularity, user group conflicts, harvest pressure and expectations of a sustainable fishery resource pose the continual need for the best science available to construct framework of management decisions. Many regulation changes have occurred in the past five decades as a reaction

to fishery issues. With the requirement of a recreational shellfish license in 2004, funds are available and ODFW shellfish staff hope to provide proactive management to eliminate future fishery issues.

EFFECT OF IN VITRO INTERACTIONS BETWEEN MERCENARIA MERCENARIA HEMOCYTES AND SEVERAL SPECIES OF HARMFUL ALGAE.

Hélène Hégaret (Department of Marine Sciences, UCONN, Groton, CT 06340), Gary H. Wikfors (NOAA-NMFS, Milford, CT 06460), Madeleine Gonçalves (IUEM-UBO, Technopole Brest-Iroise, 29280 Plouzané), Sandra E. Shumway (Department of Marine Sciences, UCONN, Groton, CT 06340)

Shellfish species are very often exposed to harmful algal blooms (HABs), which can have noxious effects. The northern quahog (=hard clam), *Mercenaria mercenaria*, can experience blooms of several HAB species, such as *Prorocentrum minimum*, *Heterosigma akashiwo* or *Alexandrium fundyense*. *In vitro* tests of interactions between those harmful algae and *M. mercenaria* hemocytes have been conducted, measuring possible differences in hemocyte parameters attributable to harmful algae and measuring also the effect of hemocytes on the algae themselves. Indeed, the purpose of these experiments was to understand the possible roles of the hemocytes in bivalve responses to HABs, and how the algal cells are affected by these responses. Using microscopic and flow-cytometric observations, changes in morphology and physiology of the hemocytes, and of the algal cells, were assessed. In hemocytes, changes in hematology and physiology, including cell concentration, mortality, phagocytosis, adhesion, apoptosis and oxidative burst response, were determined. Changes in the physiology and the characteristics of the algal cells were determined as well: mortality, size, chlorophyll fluorescence, and internal complexity. The results show differences in the hemocyte responses when exposed to the several harmful algae tested. Examples of hemocyte responses include phagocytosis of algal cells or adhesion around cells, with attendant increases in dead, achlorotic algal cells. Thus, *in vitro* tests allow a better understanding of the role of the hemocytes and the hemolymph in the defense mechanisms in molluscan shellfish to harmful algal cells.

ENVIRONMENTAL EFFECTS ON SEA URCHIN GONAD DEVELOPMENT – A PIECE OF THE PUZZLE !

Phil James (National Institute of water and Atmospheric Research)

The effects of various environmental conditions on the development of urchin gonads have been examined in relation to roe enhancement (fattening) of wild caught sea urchins (*Evechinus chloroticus*) in New Zealand.

The effects of depth and density were examined with urchins held in sea-cages suspended from a mussel longline at depths of 3 and 6 m and at high and low densities. The results showed there were no significant differences between the enhancement of roe quantity (GI) or quality (colour) in urchins held at 3 m or at 6 m, or between urchins held at low and high density.

The effects of wave and feeding disturbance were measured by holding urchins in sea-cages suspended from a surface line (wave disturbed) or subsurface buoyed from a bottom line (not wave disturbed) and fed and cleaned *in situ* underwater or removed from the water for feeding and cleaning. Increased water movement in the wave disturbed cages resulted in a higher GI in these urchins compared to urchins in cages that were sub surface buoyed. Feeding disturbance had no effect on the GI values or colour quality of the urchin gonads, regardless of the disturbance treatment.

An experiment to measure the effects of seasonality and the initial gonad condition on roe enhancement has shown that it is possible to significantly increase the amount of roe in a relatively short period throughout a 12 month period and that the initial condition (GI) of the urchins has a significant effect on the efficacy of roe enhancement.

EFFECT OF DIETARY SELENIUM ON THE SEA URCHIN LYTECHINUS VARIEGATUS



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Warren T Jones (University of Alabama at Birmingham), Dr. Mickie L Powell (University of Alabama at Birmingham), Victoria K Gibbs (University of Alabama at Birmingham), Hugh S Hammer (University of Alabama at Birmingham), Dr. John M. Lawrence (University of South Florida), Dr. Addison L. Lawrence (Texas A&M University), Dr. Stephen A Watts (University of Alabama at Birmingham)

Selenium is an essential micronutrient in the diet of many animals; however, high levels may be toxic. In mammals, selenium is an important antioxidant, working with vitamin E to protect cell membranes and prevent free radical generation. Small *L. variegatus* (ca. 15.5 g initial wet weight) were fed formulated feeds with low, medium, and high levels of selenium for 12 weeks (calculated at 0.23, 0.91, and 2.5 ppm; levels based on previously established dietary levels for other marine invertebrates, supplemented as Na₂SeO₃). Consumption was measured daily, and urchins were weighed and test diameters measured at 4-week intervals. Wet weights of individuals fed the high selenium feed were 12% lower ($p=0.006$) than those fed the low and medium levels (43.11 ± 1.21 , 43.03 ± 1.22 , and 37.81 ± 1.37 g wet weight for low, medium, and high, respectively) of dietary selenium at 12 weeks. Urchins fed the low selenium feed had higher specific growth rates between 4 and 8 weeks (1.5 vs. 1.2 and 1.1 % body wet weight gain/day for low, medium and high levels, respectively). Additionally, test diameters for urchins fed the low and medium selenium feeds were slightly higher ($p=0.077$) than those fed the high selenium (43.2 ± 0.39 , 43.2 ± 0.50 , and 41.8 ± 0.51 mm, respectively) feed. Feed consumption rates were similar among treatments suggesting weight gain was affected by other physiological mechanisms. Although no mortalities were observed, noticeable pathologies were found in the urchins fed high selenium feeds. These results suggest dietary toxicity at high levels of selenium. Supported by the Mississippi-Alabama Sea Grant Consortium.

DEVELOPMENT OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN CRASSOSTREA ARIAKENSIS AND RELATED CRASSOSTREA SPECIES

Hyungtaek Jung (University of Delaware), Woo-jin Kim (National Fisheries Research & Development Institute), Patrick Gaffney (University of Delaware)

As a candidate for deliberate introduction into Atlantic waters, the Suminoe or jinjiang oyster *Crassostrea ariakensis* has recently been the subject of intense interest, with particular focus on its ecology, taxonomy and population genetics. Because the taxonomy of Asian *Crassostrea* is still incomplete, and identification on morphological grounds difficult, a suite of genetic markers suitable for identifying species and subspecies is needed. Such markers will allow screening of candidate source material, allow development of genetic signatures of hatchery strains for evaluating introductions, and ultimately provide tools for selective breeding and pedigree monitoring. We have tested primers originally designed to amplify fragments of known genes (Type I markers) in *C. gigas* for their ability to amplify putative homologs in the related Asian species *C. angulata*, *C. ariakensis* and *C. hongkongensis*. Of 16 loci developed for *C. gigas*, all amplified successfully in *C. angulata*, 15 amplified in *C. ariakensis* and 14 amplified in *C. hongkongensis*. In contrast, only five loci could be amplified in the Atlantic species *C. virginica*. Direct sequencing of amplicons revealed multiple intraspecific and interspecific candidate polymorphisms, providing tools for genetic identification, linkage mapping, pedigree monitoring and phylogenetic analysis.

INGESTION OF MARINE AGGREGATES BY SUSPENSION-FEEDING MOLLUSCS

Dustin Kach (University of Connecticut), J.Evan Ward (University of Connecticut)

A large body of literature focuses on suspension-feeding processes of molluscs, including the efficiency of particle capture. Bivalves, for example, capture individual picoplankton cells (0.2 - 2.0 μm) with a retention efficiency of less than 50%, leading to the assumption that such particles are not an important food resource. Picoplankton, however, are often concentrated within aggregates of much larger size. This study builds on our preliminary results and further investigates the ability of suspension feeders to ingest pico-particles (< 2 μm) bound in aggregates. We fed the mussel, *Mytilus edulis*, the scallop, *Argopecten irradians*, and the slipper snail, *Crepidula fornicata*, 1 μm fluorescent beads that were (1) evenly dispersed in seawater, or (2) embedded within laboratory-made

aggregates. Dispersed 10 μm beads were also delivered so that feeding activity could be determined. All feces were collected over 48 hr period, digested in NaOH, and the fluorescent activity measured. The number of beads ingested was then calculated using a previously established standard curve. Results indicate that aggregates significantly enhance the ingestion of 1 μm beads by all three animals. Differences among species in their ability to ingest aggregates and pico-particles, however, were evident. When feeding on aggregates, mussels demonstrated a ten fold increase in the number of 1 μm beads ingested. Compared to mussels, scallops ingested fewer aggregates, and slipper snails ingested more dispersed beads. These differences may be a consequence of variations in gill structure and mechanisms of particle processing. Our data suggest that aggregates allow suspension feeders to utilize embedded particles as food.

IDENTIFICATION OF GROWTH BANDS IN COCKLE, *FULVIA MUTICA* SHELLS IN KOREA

Do-hyung Kang (Cheju National University, Republic of Korea), Heung-sik Park (Korea Ocean Research & Development Institute), Chris A. Richardson (University of Wales, UK), Kwang-sik Cho i (Cheju National University, Republic of Korea)

This study reports identification of annual growth bands recorded in shell from cockle, *F. mutica* (Bivalvia: Cardiidae) collected from Jukdo Korea. The growth lines were identified using acetate peel replicas of the shell sections. Total number of growth lines and distance (i.e. growth interval) between inter-growth lines were assessed using image-processing software. Age of the cockle was estimated by counting the number of each dark band in sectioned shell and umbo. Periodicity of the light and dark bands was compared with the daily water temperature data of the sampling area retrospectively. A total of 790 growth lines were identified from the umbo. Age of the cockle used in the analysis was predicted to be 6.5 year. The maximum distance (23.4 μm) and the minimum distance (1.2 μm) were found in a 3-year-band and in a 6-year-band, respectively. Average distance of the cockle was highest in 3-year-band. Periodicity of the bands was closely corresponded with temperature data, suggesting that retrospective technique was suitable for aging of the cockle.

COULD LIMNOPERNA FORTUNEI BE WORSE THAN DREISSENA POLYMORPHA? POTENTIAL SPREAD AND ECOSYSTEM IMPACTS

Alexander Y. Karatayev (Stephen F. Austin State University, Nacogdoches, TX), Demetrio Boltovskoy (Universidad de Buenos Aires, Argentina), Dianna K. Padilla (Stony Brook University, Stony Brook, NY), Lyubov E. Burlakova (Stephen F. Austin State University, Nacogdoches, TX)

Although taxonomically unrelated, the zebra mussel (*Dreissena polymorpha*) and golden mussel (*Limnoperna fortunei*) have very similar life histories, suggesting that similar vectors are involved in their spread. Both are sessile, byssate bivalves with a planktonic larval stage and high reproductive capacity. Adults of both species attain extremely high densities physically changing the substrate, and because they are such effective suspension feeders they greatly enhance benthic-pelagic coupling and act as powerful ecosystem engineers. We compare environmental limits and ecological impacts of the well studied *D. polymorpha* with the lesser known *L. fortunei* to predict the potential spread and ecosystem impacts of the golden mussels. *Limnoperna fortunei* reaches population densities similar to or higher than *D. polymorpha*. It has comparable filtering rates and therefore may have similar or even stronger ecosystem impacts. In addition *L. fortunei* has broader environmental tolerance, including to high temperatures, low pH, low calcium content and water pollution. Therefore, *L. fortunei* may be much more successful than *D. polymorpha* in regions dominated by acidic, soft and contaminated waters. We predict that in the near future *L. fortunei* will colonize the southern and central parts of North America, Southern Europe, Northern Africa, and Australia, as well as expand its distribution in South America and Asia. Although to date *D. polymorpha* is considered the most aggressive freshwater invader, soon many waterbodies that are already affected by *D. polymorpha* may get another, even more aggressive invader.

ENHANCED BLUE CRAB ABUNDANCE ON RESTORED OYSTER REEFS



**Abstracts of the 98th Annual Meeting
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M. Lisa Kellogg (University of Maryland), Christopher McIntyre (Coastal Carolina University), Kennedy C. Paynter (University of Maryland), Kennedy T. Paynter (University of Maryland)

In recent years, the goals of oyster reef restoration in Chesapeake Bay have shifted from simply enhancing the oyster fishery to restoring the ecosystem services that were once provided by healthy oyster reefs. Like historical reefs, restored oyster reefs produce topographically-complex reef structures that enhance both the abundance and diversity of the associated macrofaunal community. Restored oyster reefs, therefore, have the potential to provide highly-mobile organisms such as the blue crab, *Callinectes sapidus*, with both an abundant supply of prey and a refuge from predation. To determine whether restored oyster reefs are a preferred habitat for blue crabs, blue crab populations were estimated on restored oyster reefs and adjacent control areas at two restoration sites in the South River: Glebe Bay and Duvall Creek. On each sampling day, one 50-m trotline was laid on the restored reef and another was placed on an adjacent unrestored control area. Alternating between the two lines, each line was sampled 10 times. All crabs caught were retained to avoid recapture of the same individuals. More crabs were caught on the restored reefs than the control sites at both Glebe Bay ($p = 0.004$) and Duvall Creek ($p = 0.037$). Assuming these capture rates represent relative crab abundances, these preliminary findings suggest that blue crab densities may be higher on restored oyster reefs. However, additional studies will be necessary to determine why blue crabs prefer the restored reefs, and whether restored reefs enhance blue crab production.

**DEVELOPMENT OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN THE PACIFIC OYSTER
CRASSOSTREA GIGAS**

Woo-jin Kim (National Fisheries Research and Development Institute, Korea), Hyungtaek Jung (University of Delaware), Patrick Gaffney (University of Delaware)

Single nucleotide polymorphisms (SNPs) are useful for characterizing population structure, for genetic mapping, and as a tool for pedigree monitoring and marker-assisted breeding schemes. Our objective is to develop SNP markers for the Pacific oyster *Crassostrea gigas*, the most widely cultured bivalve species. Primers were designed based from expressed sequence tags (ESTs) to amplify small fragments (200-400 bp) spanning the 3' end of coding sequence and the 5' end of the 3' UTR. Most (22 of 24) primer pairs successfully amplified PCR products from genomic DNA. Direct sequencing of amplified targets resulted in the identification of 321 candidate SNPs within 22 identified nuclear genes. The observed frequency of SNPs in *C. gigas* was 4.32 per 100 bp: 1.94 in exons, 9.06 in introns, and 4.64 in the 3' UTR. Individuals from an inbred hatchery line showed reduced polymorphism. Oysters from three populations of *C. gigas* (Korea, Hokkaido, Kyushu) and one *C. angulata* population (Portugal) could be differentiated using estimated genetic similarity based on SNP allele-sharing at ten loci. All loci were successfully amplified from *C. angulata*, underscoring its close genetic relationship to *C. gigas*. The majority of identified SNPs differed between the two species, providing multiple independent markers for identification of both species and hybrids, and for marker-assisted introgressive hybridization. Mapping of these loci is in progress. The SNP markers developed in this study will enable researchers to elucidate population structure in the native range of the two species, as well as the characterization of cultured lines.

**RELATIONSHIPS AMONG PARASITES AND PATHOLOGIES IN SENTINEL BIVALVES: NOAA STATUS
AND TRENDS 'MUSSEL WATCH' PROGRAM**

Yungkul Kim (Haskin Shellfish Research Laboratory, Rutgers University), Eric N. Powell (Haskin Shellfish Research Laboratory, Rutgers University)

NOAA's National Status and Trends 'Mussel Watch' Program includes a comprehensive survey of the histopathology of sentinel bivalves from the East, West, Gulf, and Great Lakes coasts of the United States. We analyzed the data for 1995-1998 to identify relationships between various parasites, various pathologies, and between parasite/pathology pairs with the goal of identifying consistencies and differences in these relationships between sentinel bivalves and between major geographic units of the U.S. coastline. The prevalences of parasite, pathology, and parasite-pathology pairs were significantly correlated more frequently for oysters than for mussels. The number of significant correlations within Gulf-coast oysters exceeded the number within East-coast oysters. Correlations were least frequent among East-coast mussels. The incidence of significant negative correlations in

prevalence far exceeded the incidence of significant positive correlations in all species and bay regions. Significant relationships in infection intensity occurred much less frequently than for prevalence. Positive correlations occurred more frequently than they did for prevalence. Both trends reinforce the belief that environmental factors controlling transmission are likely distinctive from those controlling proliferation. Only a few relationships between parasites were common to more than one sentinel bivalve or more than one coastal region. Not a single common relationship involved a pathology. However, though commonalities were few, consistent trends in prevalence between mussels and oysters and between coastal regions suggest potentially important large-scale trends among some important parasite groups, particularly the gregarines and gut ciliates, gill gregarines and cestodes, prokaryotic inclusions and trematode metacercariae, and *Perkinsus marinus* and the gregarines.

MEASUREMENT OF SCOPE-FOR-GROWTH IN FRESHWATER MUSSELS AND THE RELEVANCE FOR WATER QUALITY, ECOSYSTEM FUNCTION AND BIOMONITORING

Danielle A. Kreeger (Partnership for the Delaware Estuary)

Native freshwater mussels continue to garner increasing attention for their biodiversity plight, ecological value, and usefulness for biomonitoring. Despite the rising chorus, many key aspects of their basic biology remain poorly studied. Of particular note is the lack of attention to organism-level physiological functions that represent the nexus, or “missing link,” essential to relating environmental conditions to population- and species-level fitness, and vice versa. Small spatial and temporal shifts in environmental conditions, food quantity and quality, or animal nutritional demands can elicit significant shifts in organism-level processing rates and fitness, with concomitant effects to the ecosystem where populations remain abundant. Decades of research with marine species have yielded an arsenal of powerful methods to quantify spatial, temporal, intraspecific and interspecific variation in physiological rates and status. And yet, only one physiological rate metric (clearance) and one status metric (condition) have been widely adopted for freshwater mussels, and their meaning has at times been misinterpreted. In this presentation I will briefly review the available “tool kit,” describe methods needed to calculate scope-for-growth, and discuss the proper interpretation of these and other physiological metrics as being context specific, rather than absolute. Specifically, I will present case study data for the allometric rates of clearance, defecation, excretion and respiration of species from both the Atlantic and Pacific slopes. When standardized and understood, physiological methods and information will play a pivotal role in monitoring and optimizing the health of captive animals, quantifying the functional importance of mussel populations in nature, and biomonitoring the status of our waterways.

TRANSPARENT EXOPOLYMER PARTICLES (TEP) FROM SUSPENSION FEEDERS AND THEIR EFFECTS ON PARTICLE AGGREGATION

Binglin Li (University of Connecticut), J. Evan Ward (University of Connecticut)

Transparent exopolymer particles (TEP) can form from dissolved precursors released by phytoplankton. Recent studies have also demonstrated that benthic suspension feeders, such as bivalves, can contribute significantly to the TEP pool in coastal waters. Whereas TEP from phytoplankton is important for the aggregation of suspended material, the role of TEP from suspension feeders on aggregate formation is unknown. The purpose of this study was to test the hypothesis that organic compounds, including TEP, released from suspension feeders enhance the formation of particle aggregates in coastal waters. In laboratory experiments, TEP was collected from blue mussels (*Mytilus edulis*) and sea vases (*Ciona intestinalis*) using previously established methods. One of two particle types, silica-amino beads or diatomaceous earth (ca. 10 μ m), were then added to seawater with and without TEP produced by the animals. Bottles (250 ml) containing suspended particles were placed on a roller table and rolled for 48 hours. Changes in the size distribution of particles in each jar were followed over time by means of an electronic particle counter, and aggregates larger than 100 μ m were counted using a rafter cell and microscope. Dissolved and total organic carbon, and bacterial concentrations were determined before and after the rolling period. Results indicate that significantly more particle aggregates are produced in seawater containing TEP from suspension-feeding animals than in control water. Aggregation of both particle types was similarly enhanced. Our results suggest that

organic material released by suspension-feeders can enhance aggregate formation and hence the deposition of material to the benthos.

VARIANCE IN REPRODUCTIVE SUCCESS OF THE FLAT OYSTER *OSTREA EDULIS* L. ASSESSED BY MICROSATELLITE-BASED PARENTAGE ANALYSES

Delphine Lallias (Ifremer - La Tremblade, France), Nicolas Tairs (Ifremer - La Tremblade, France), Pierre Boudry (Ifremer - La Tremblade, France), François Bonhomme (UMR5171 Ifremer-CNRS-Université Montpellier II, Sète, France), Sylvie Lapègue (Ifremer - La Tremblade, France)

The European flat oyster (*Ostrea edulis* L.) is a marine bivalve whose natural geographical distribution ranges along the European Atlantic coast from Norway to Morocco, in addition to the Mediterranean and Black Sea. Previous studies of allozymes, microsatellites and mitochondrial differentiation over the whole range concluded that a significant divergence existed between Mediterranean and Atlantic populations, together with an isolation-by-distance pattern. However, the average mitochondrial haplotypic diversity displayed a high among populations variance, reflecting smaller effective population size in some locations. Additionally, a ten-fold quantitative difference was observed in the same study in *Fst* between the mitochondrial and the nuclear genomes, which could be due to sex biased differential reproductive success between males and females. In order to further document this hypothesis, two experiments were conducted. First, brooding females were sampled in a wild population and the number of males fertilizing a given female estimated. Then, parentage analyses were achieved under experimental conditions: successive mass spawnings were collected from a population of potential genitors kept in hatchery, whose genotypes were known, in order to infer a posteriori the relative contribution of each genitor. Moreover, we aimed to better understand the reproduction dynamics of this species. Several patterns of spawning could be distinguished: unique, successive or extended in time. The different parental contributions and reproductive behaviors observed in these experiments are discussed in the context of the hypothesis of a variance in the reproductive success of males and females and consequences in local and temporal reduced effective population sizes.

TRANSCRIPTOME ANALYSIS OF PACIFIC OYSTER *CRASSOSTREA GIGAS* FAMILIES EXPOSED TO HEAT STRESS

Paul Lang (Oregon State University, Dept. of Fisheries and Wildlife), Christopher J. Langdon (Oregon State University, Dept. of Fisheries and Wildlife), Mark D. Camara (United States Department of Agriculture, ARS Program)

We studied the transcriptome level response of oysters to heat stress using microarrays developed by the Oyster Microarray Consortium to compare gene expression among selectively-bred oyster families that differed in sensitivity to heat stress. Spat (n=100 each per family) of 53 oyster families were exposed to 43°C water for 1 h. We then assessed mortality 2-d intervals for 6 d. Survival ranged from 6% to 85% and we classified families with >70% survival as resistant those with 30 - 70% survival as moderately resistant, and those with <30% survival as sensitive. Animals from the four highest- and the four lowest-surviving families were reared to 2 years of age and tested again. Survival ranged from 0.03% to 95% for adults. Four of the eight families retained their classification of heat-resistant or heat-sensitive, and the remaining four families were re-classified as moderately heat sensitive.

For transcriptome analysis, we exposed the two resistant families and two susceptible families to a non-lethal heat shock (40°C for 1 h) and collected gill tissue from three animals per family at 1h, 3h, 6h, and 24h following heat shock. We pooled tissue samples within family for each sampling time and extracted total RNA from each pooled sample. We labeled each RNA sample with a fluorescent dye, and hybridized the sample to a single microarray chip. Fluorescence data was collected using a ScanArray Express instrument. Genes that underwent coordinated

changes in expression level were clustered into groups using multivariate exploratory analysis. The results of these analyses will be discussed.

IMPROVEMENT IN YIELDS OF THE PACIFIC OYSTER *CRASSOSTREA GIGAS* AFTER TWO GENERATIONS OF SELECTION.

Chris Langdon (Hatfield Marine Science Center, Oregon State University), Ford Evans (Hatfield Marine Science Center, Oregon State University), Sean Matson (Hatfield Marine Science Center, Oregon State University), Drew Mosher (Hatfield Marine Science Center, Oregon State University), Alan Barton (Hatfield Marine Science Center, Oregon State University)

Globally, the Pacific oyster is the most valuable aquaculture species, with a harvest of 4.2 million metric tons valued at \$3.5 billion (FAO Fisheries Statistics 2002). The West Coast is the biggest regional producer of oysters in the U.S. with a wholesale value of \$68 million. In contrast to many other U.S. agricultural commodities, there had been no long-term, funded research program to select and manage Pacific oyster broodstock for enhanced production. In response to this need, the Molluscan Broodstock Program (MBP) was established in 1995 to implement a selective breeding program to improve broodstock and increase commercial production of Pacific oysters on the West Coast, U.S. Since MBP's inception, about 1300 oyster families have been planted and evaluated at commercial grow-out sites from Alaska to California, U.S. Results show that after two generations of selection, the average yield of oyster families derived from selected MBP broodstock is 29% greater than that of families from "wild" unselected broodstock and 34% greater than that of industry broodstock. Furthermore, the five top-performing families (recommended for commercial hatcheries) from F2 MBP cohorts have an average yield that is 77% greater than that of families from industry broodstock. MBP broodstock are used in commercial hatcheries for large-scale seed production.

THE POTENTIAL ROLE OF FRESHWATER MUSSELS IN REDUCING GIARDIA AND OTHER HUMAN PATHOGENS IN WATERSHEDS OF THE PACIFIC NORTHWEST

Chris Langdon (Hatfield Marine Science Center, Oregon State University), David Close (Confederated Tribes of the Umatilla Indian Reservation), Peggy Bradley (Institute of Simplified Hydroponics)

Freshwater mussels were abundant in rivers of the Pacific Northwest and were an important source of nutrition for many Native Americans in pre-European times. Since then, populations of freshwater mussels have declined. Habitat changes, pollution and declines in salmon (an important upstream transport vector of larval stages) are likely causes. These declines will result in a reduction in the filtering capacity of mussel populations and removal of suspended sediments and organisms, including the human pathogens *Giardia duodenalis* and *Cryptosporidium parvum*.

Laboratory studies showed that adult (91-127 mm shell length) freshwater mussels (*Margaritifera falcata*) filtered mice-derived *Giardia* (*G. muris*) at rates of 0.49 l h⁻¹ g⁻¹ mussel dry weight. In addition, mussels filtered microalgae [*Schizochytrium* sp.; in the same size range as *G. duodenalis* (2.7 – 7.1 μm)] at rates of 0.39 to 0.41 l h⁻¹ g⁻¹ dry weight. The filtering action of mussels could be an important natural mechanism for reducing concentrations of human pathogens in streams and rivers.

A LEAST COST FORMULATED FEED FOR GONAD PRODUCTION OF THE SEA URCHIN *LYTECHINUS VARIEGATUS*

A.L. Lawrence (Texas A&M University System, Port Aransas, Texas 78373), S.A. Watts (University of Alabama, Birmingham, Birmingham, Alabama 35294), M.L. Powell (University of Alabama, Birmingham, Birmingham, Alabama 35294), J.M. Lawrence (University of South Florida, Tampa, Florida 33620)

Feeds are critical to successful aquaculture production. Feed usually represent 30% to 50% of the variable cost. Dry pelleted semi-purified feeds have recently been used to produce roe of marketable quality for *Lytechinus*

variegatus. Using ingredient and nutrient limits for semi-purified feeds and substituting practical ingredients for purified ingredients such as whole wheat grain for wheat starch and dehulled, defatted soybean meal (48% protein) for purified isolated soy protein, a commercial urchin feed was formulated using a least cost program. Calculated feed levels for crude protein, soluble carbohydrate, crude fiber, total ash and crude fat were 25%, 34.9%, 2.9%, 19.5%, and 7.5%, respectively, with an ingredient cost of \$609.90/M.T. Protein was the most expensive nutrient with feed costs decreasing to \$557.70 and increasing to \$659.80/M.T. for 15% and 35% protein feed levels, respectively. Marine (e.g. kelp, fish) and lipid (e.g. carotenoid, cholesterol) ingredients represented the largest ingredient costs. Estimating feed mill administrative, amortization, production and margin is approximately \$200/M.T., the selling price for 25% protein commercial urchin feed would be \$809.90/M.T. Using shrimp raceway and feed technology and economic data and data obtained last year by the University of Alabama at Birmingham and Texas A&M System, urchin aquaculture for roe production for human consumption has a larger potential margin than intensive shrimp production in raceways. Supported by Mississippi-Alabama Seagrant and Project No RI-9125, Texas Agricultural Experiment Station.

SIZE-SPECIFIC SURVIVAL AND FISHING MORTALITY ESTIMATES FOR RED ABALONE, *HALIOTIS RUFESCENS*, USING MARK-RECAPTURE DATA

Robert Leaf (Virginia Tech), Laura Rogers-bennett (Bodega Marine Lab and California Department of Fish and Game), Peter L. Haaker (California Department of Fish and Game)

Estimates of size specific natural and fishing mortality are currently not available for red abalone, *Haliotis rufescens*, in northern California. We examined annual survivorship of three size classes (< 100 mm, 100.1 to 178 mm, and > 178 mm, the legal fishing limit) of red abalone using capture-mark-recapture data in northern. We estimate fishing mortality comparing fished and reserve sites in northern California. The number of tagged individuals (n = 273 to 2,145), survey occasions (n = 3 to 7), and size composition of individuals (41.5 to 227 mm, maximum shell length, MSL) were variable at each site. The annual survival probabilities of the smallest size class (< 100 mm MSL) at one reserve site in northern California was $0.52 \text{ y}^{-1} \pm (0.05 \text{ SE})$ and was $0.36 \text{ y}^{-1} \pm (0.07 \text{ SE})$ to $0.51 \text{ y}^{-1} \pm (0.08 \text{ SE})$ in southern California. Annual survival of the mid-size class (100.1 to 178 mm MSL) from four sites at northern California ranged from $0.47 \text{ y}^{-1} \pm (0.05 \text{ SE})$ to $0.71 \text{ y}^{-1} \pm (0.04 \text{ SE})$. The largest size class (>178 mm MSL) had a large range of annual survivorship values of $0.26 \text{ y}^{-1} \pm (0.06 \text{ SE})$ to $0.95 \text{ y}^{-1} \pm (0.08 \text{ SE})$ in fished and reserve sites in northern California. Instantaneous fishing mortality (F) was estimated to be 0.68 to 1.29. This study is an example of how capture-mark-recapture data from fished and reserve sites and the selection of parsimonious models can be used to estimate natural and fishing mortality estimates.

PRELIMINARY VALIDATION OF AGE-AT-LENGTH OF RED ABALONE, *HALIOTIS RUFESCENS*, USING BOMB RADIOCARBON

Robert T. Leaf (Virginia Tech), Allen H. Andrews (Moss Landing Marine Laboratories), Gregor M. Cailliet (cailliet@mlml.calstate.edu)

Current methods to describe the age-at-length relationship of red abalone (*Haliotis rufescens* Swainson) are insufficient to determine the ages of large individuals because their shell lengths exceed those predicted by model L_{∞} values. We evaluated the utility of analyzing atomic bomb generated radioisotope ^{14}C in shell as a method to validate the age-at-length relationship of *H. rufescens*. Fabens' (1965) method was used to determine von Bertalanffy growth function (VBGF) parameters ($L_{\infty} = 313 \text{ mm}$, $k = 0.051 \text{ y}^{-1}$ (0.042 to 0.059, 95% CI)) based on data from a multi-year, multi-site tag-recapture study. Shell carbonate was sampled at four locations on a single shell (251 mm, maximum shell length (MSL)) with the objective to bracket the rise of the radiocarbon signal, known to occur during ~1957 in the NE Pacific. There was close correspondence to the radiocarbon values of extracted shell carbonate and estimated dates of formation based on VBGF estimates. The 251 mm (MSL) red abalone specimen was predicted to be 27 to 38 years old. This study presents preliminary results from radiocarbon analysis of shell carbonate and demonstrates the utility of validating the age-at-length relationship of red abalone with this method.

THE EFFECTS OF ANTI-FOULING TREATMENTS FOR *STYELA CLAVA* ON LONG-LINE CULTURED *MYTILUS EDULIS* IN PRINCE EDWARD ISLAND, CANADA

Neil Leblanc (Atlantic Veterinary College), Jeffery Davidson (Atlantic Veterinary College), Mary Mcniven (Atlantic Veterinary College), Thomas Landry (Fisheries and Oceans), Rejean Tremblay (University of Quebec)

Currently, the mussel industry in Prince Edward Island is facing serious production problems associated with an invading species. *Styela clava* or clubbed tunicate is an invading species found in Prince Edward Island in the late nineteen nineties. It is a sessile filter feeder, which forms dense colonies on mussel lines causing several serious production problems. In the first few bays found to contain this organism the mussel farms were overwhelmed by this invader. The objective of this experiment was to determine the effect that acetic acid treatments, used to get rid of the tunicates, has on the mussels being farmed.

A field trial was conducted in November 2002 involving ½ inch socked juvenile mussels. The trial involved two acetic acid treatments, a thirty second and two-minute dip in 5% acetic acid. The treated and control socks were then placed systematically on a longline in New London Bay, Prince Edward Island from north to south in the order two-minute acetic acid dip, control, thirty second acetic acid dip.

Seven months later, in June 2003, the line was retrieved. Studies in the lab consisted of weighing the entire sock, two-foot sections, measuring 500+ specimens per treatment for length, performing a condition index experiment on thirty specimens of each treatment, and allozyme analysis on fifty individuals per treatment to determine heterozygosity. This experiment indicates that acetic acid can be fatal to mussels but does not cause serious longterm health problems. These factors should be taken into account when devising methods and dosages for the anti-fouling treatments.

SEED QUALITY ASSESSMENT OF *MYTILUS EDULIS* STOCKS IN THE GULF OF ST. LAWRENCE, CANADA

Neil Leblanc (Coastal Zones Research Institute), Fabrice Pernet (Coastal Zones Research Institute), Rejean Tremblay (University of Quebec), Thomas Landry (Fisheries and Oceans), Jeff Davidson (Atlantic Veterinary College)

Seed supply is a crucial part of any aquaculture operation and the mussel industry in eastern Canada is no exception. This industry relies solely on wild spat collection to furnish seed for its aquaculture operations. High mortality in cultured mussels has periodically been a problem in the Gulf region and this has sometimes been attributed directly to the seed stock that has been used. This has occurred in the absence of a pathological explanation and studies on mussel stocks in the Gulf region have pointed to the genetic composition of mussel populations as a significant factor contributing to large scale mortality events.

The objective of the study was to develop and assess seed quality criteria for *Mytilus edulis*. We have taken mussel seed from six different locations in two provinces in the Gulf region and performed a reciprocal transfer among the seed source sites creating a 6 x 6 (seed x location) field study using a total of 3240 individually tagged mussels. Productivity was measured using the variables growth, survival, and condition index. Explanatory variables included lipid content and composition, heterozygosity level, microsatellites loci characterization, histology and oxygen consumption.

**MINING EST DATABASE FOR SINGLE-NUCLEOTIDE POLYMORPHISMS IN THE EASTERN OYSTER
(CRASSOSTREA VIRGINICA)**

Jeong-ho Lee (Haskin Shellfish Research Laboratory, Rutgers University), Ximing Guo (Haskin Shellfish Research Laboratory, Rutgers University)

Single-nucleotide polymorphisms (SNPs) are important resources in genomic analysis, particularly when they are derived from expressed sequence tags (ESTs). SNPs derived from EST are excellent markers for genetic mapping because of their representation of functional genes and potential for high throughput genotyping. SNPs can be developed by re-sequencing or by mining existing sequence databases. The public EST databases represent an enormous but heterogeneous repository of sequences, including many from a broad selection of oyster species and varieties. The significant redundancy within large EST collections makes them an attractive resource for rapid identification of SNPs. At the present time, only a limited number of SNPs are available in the eastern oyster (*Crassostrea virginica*). In this study, we used a bioinformatics strategy to discover SNPs within an eastern oyster EST database. A collection of >9,000 eastern oyster ESTs was downloaded from GenBank and assembled into 602 contiguous sequences (contigs). The contigs were then visually inspected to identify SNPs with high minor allele frequencies and good flanking sequences. A total of 206 candidate SNPs from 100 genes were identified and characterized. Polymorphism information content for the SNPs ranged from 0.11 to 0.50. They are being validated and mapped in the eastern oyster.

**EFFECTS OF THE DINOFLAGELLATE KARENIA BREVIS ON LARVAL DEVELOPMENT IN THREE
SPECIES OF BIVALVE MOLLUSC FROM FLORIDA**

Jay R. Leverone (Mote Marine Laboratory; University of South Florida), Norman J. Blake (University of South Florida), Sandra E. Shumway (University of Connecticut)

The effects of *Karenia brevis* (Wilson clone) on larval survival and development of the bay scallop, *Argopecten irradians*, northern quahog, *Mercenaria mercenaria*, and eastern oyster, *Crassostrea virginica* were studied in the laboratory. Larvae were exposed to cultures of whole and lysed cells, with mean total brevetoxin concentrations of 53.8 and 68.9 $\mu\text{g} \cdot \text{L}^{-1}$, respectively. Survival of early (3-day-old) larvae was generally over 85% for all shellfish species at *K. brevis* densities of 100 cells $\cdot \text{ml}^{-1}$ or less, and not significantly different between whole and lysed culture. At 1,000 cells $\cdot \text{ml}^{-1}$, survival was significantly less in lysed culture than whole culture for both *M. mercenaria* and *C. virginica*. Survival of late (7-day-old) larvae in all three species was not significantly affected by *K. brevis* densities of 1,000 cells $\cdot \text{ml}^{-1}$ or less. At 5,000 cells $\cdot \text{ml}^{-1}$, however, survival was reduced to 37, 26 and 19% for *A. irradians*, *M. mercenaria* and *C. virginica*, respectively. Development of *C. virginica* and *M. mercenaria* larvae was protracted at *K. brevis* densities of 1,000 cells $\cdot \text{ml}^{-1}$ and greater. These results suggest that blooms of *K. brevis*, and particularly their associated brevetoxins, may have detrimental consequences for Florida's shellfisheries by disrupting critical larval processes. Special attention should be paid to blooms of *K. brevis* where these shellfish naturally occur or where aquaculture and restoration activities are either ongoing or planned.

**RESPONSE OF BAY SCALLOP POPULATIONS TO LARVAL RELEASES AND AN UPDATE ON CURRENT
RESTORATION ACTIVITIES IN PINE ISLAND SOUND, FL**

Jay R. Leverone (Mote Marine Laboratory; University of South Florida), Stephen P. Geiger (Florida Fish and Wildlife Research Institute), William S. Arnold (Florida Fish and Wildlife Research Institute), Jaime M. Greenawalt (Sanibel-Captiva Conservation Foundation)

In 2003, we undertook a novel restoration approach to enhancing bay scallop populations in Pine Island Sound, Florida. This approach involved releasing competent, late-stage larvae into containment booms which isolated the water column, thereby preventing the larvae from excessive dispersion. Three booms were used for larval releases and an additional boom served as a control (no larvae added). Larval settlement and juvenile recruitment were monitored throughout the following year. Scallop spat recruited to artificial substrates in all three

treatments. Juvenile scallops, surveyed in Feb 2004, were found at all treatment locations while being absent from the control. Adult scallops, surveyed in Jul 2004, were two orders of magnitude greater **at the restoration site** than the resident scallop population within Pine Island Sound. Adult scallops were again surveyed in Jun 2005. That year, **the entire survey area** experienced a 100-fold increase in bay scallops from the previous year. Pine Island Sound had the highest abundance of bay scallops than any other Florida estuary in 2005. These results demonstrate that controlled release of competent larvae is a viable method for ultimately restoring bay scallop populations in Pine Island Sound. This fall, our restoration efforts expanded to South Pine Island Sound, a region that has also experienced reduced scallop densities over the past several decades. We will discuss some of the potential problems associated with restoration success at this location, including freshwater inputs and reduced salinity. We are also conducting or planning larval releases in three other Florida Gulf Coast estuaries (Sarasota Bay, Boca Ciega Bay and St. Andrews Bay) during 2005-2006.

CHARACTERIZATION OF NRAMP IN PERKINSUS MARINUS AND CRASSOSTREA VIRGINICA

Zhouer Lin* (COMB, UMBI, University of Maryland, Baltimore, MD 21202, USA), José A. F. Robledo (COMB, UMBI, University of Maryland, Baltimore, MD 21202, USA), Gwendolyn Mullen (Morgan State University Graduate School Science Education), Gerardo R. Vasta (COMB, UMBI, University of Maryland, Baltimore, MD 21202, USA)

Nramp (natural resistance-associated macrophage protein) is a divalent cation transporter demonstrated to be a determinant of resistance/susceptibility to intracellular pathogens by preventing pathogens from acquiring divalent metals. Reciprocally, most parasites have developed efficient mechanisms for metal acquisition from their hosts. After characterizing Nramp in the oyster parasite *Perkinsus marinus* (*PmNramp1*), we identified two additional Nramp species in our ongoing *Perkinus* genome project (<http://www.tigr.org/tdb/e2k1/pmg/>, Microbial Genome Initiative, NSF). Neither intron boundaries nor intron numbers appear to be conserved: 7, at least 4, and 13 introns in *PmNramp1*, *PmNramp2*, and *PmNramp3*, respectively. *PmNramp1* and *PmNramp2* are more similar between them than with respect to *PmNramp3*, so far *PmNramp1* and *PmNramp3* appear to have a TATA-less promoter and the three *PmNramp* are expressed in *P. marinus* cultured trophozoites. We have found several polymorphic sites in *PmNramp1* and we are assessing their potential contribution(s) as virulence factors in the protozoan-oyster interaction. We are also characterizing *Crassostrea virginica* Nramp (*CvNramp*); *CvNramp* messages were identified in samples from hemocytes, mantle, gill, muscle, and heart. Functional and immunolocalization studies of *PmNramp* and *CvNramp* are underway (Supported by Grant ICB-0321417, NSF).

HYBRIDIZATION BETWEEN TWO GENERA OF CLAMS, SPISULA SOLIDISSIMA AND MULINIA LATERALIS, AND EARLY GROWTH TRIALS

Scott Lindell (Marine Biological Laboratory), Bethany Walton (Marine Biological Laboratory), Janice Simmons (Marine Biological Laboratory), Steven Roberts (Marine Biological Laboratory)

The Atlantic surf clam (*Spisula solidissima*) and the coot clam (*Mulinia lateralis*) occupy different habitat niches along the eastern North American seaboard. The Atlantic surf clam supports a multi-million dollar fishery and has been the subject of pilot-scale commercial aquaculture. Among the interesting properties of the coot clam that have made it useful to biologists are its ease of culture, short generation time, and high reproductive rate. Despite their apparent geographic and reproductive isolation we have cross-bred these two species in the lab. Larvae of coot clams and both hybrids (*Spisula* eggs x *Mulinia* sperm and *Mulinia* eggs x *Spisula* sperm) metamorphosed at 10 days at 20°C and were then reared on set screens and in sand substrate. Results of the first four months of growth of the hybrids and *Mulinia lateralis* will be presented here. We also present information about genetic markers for distinguishing hybrids from their parents. The results have implications for reassessing the taxonomy of mactrid clam species, and may hold promise for a new aquaculture candidate.

DOES PERKINSUS MARINUS SECRETE EICOSANOIDS?

Eric D. Lund (Virginia Institute of Marine Science), Fu-lin E. Chu (Virginia Institute of Marine Science), Ellen Harvey (Virginia Institute of Marine Science)

Perkinsus marinus, a protozoan parasite of the Eastern oyster, *Crassostrea virginica*, causes high mortalities in its host along the Atlantic and Gulf coasts of North America. Several virulence factors of *P. marinus* have been identified, but the mechanisms whereby *P. marinus* overwhelms the host immune response remain poorly understood. One class of virulence factors that has been identified in several protozoan parasites of mammals is eicosanoids, oxygenated metabolites of fatty acids. Previously we have demonstrated that *P. marinus* synthesizes a wide array of saturated, monounsaturated and polyunsaturated fatty acids, including arachidonic acid, a precursor to several eicosanoids including prostaglandins D₂, E₂ and F_{2a}. To determine if *P. marinus* produces eicosanoids in vitro meronts were cultured in a defined, lipid-free medium supplemented with cholesterol and screened for eicosanoids. GC/MS analysis of a derivatized extract of 4X10⁸ *P. marinus* meront cells revealed a peak with a spectrum similar to that of derivatized prostaglandin D₂ (PG D₂), but no other derivatized eicosanoids were detected. Subsequent enzyme immunoassay (EIA) analyses of medium from *P. marinus* meront cultures (5-7 X 10⁵ cells/ml) confirmed the presence of PG D₂ at concentrations between 40 and 140 pg/ml. These levels are comparable to those reported in the literature for *Trypanosoma* and *Plasmodium* species. The function of PG D₂ secreted by *P. marinus* is not currently known. However, prostaglandins synthesized by protozoan parasites of mammals have been shown to be immunosuppressive. Further research is needed to determine if PG D₂ inhibits oyster immune responses.

ENERGY AND PROTEIN REQUIREMENTS FOR MAINTENANCE AND GROWTH IN THE SEA URCHIN (PARACENTROTUS LIVIDUS).

Ingrid Lupatsch (National Center for Mariculture Eilat Israel), Esther Golombek (National Center for Mariculture Eilat Israel), Muki Shpigel (National Center for Mariculture Eilat Israel)

A novel approach to determining the protein and energy requirements in sea urchin is described here quantifying the requirements for energy and protein as the sum of the daily requirements for maintenance and growth.

Two growth trials of each 91 and 95 days were performed at average temperatures of 22^oC using sea urchins of 6.8g and 12.4g respectively. The digestibility of the formulated diet was determined beforehand to contain 190mg digestible protein and 14.4 kJ digestible energy per g feed. The requirement of digestible energy and protein for maintenance and growth were determined by feeding *Paracentrotus lividus* increasing levels, starting at zero and going up to apparent maximum feed intake. Total energy and protein gain in the urchins were measured by comparative slaughter technique. Protein content of whole live animals ranged from 3.7% initially up to 6.0% at the maximum feeding level corresponding to a gonadosomatic index (wet/wet) of 3.0 to 25.0 respectively.

The relationship between digestible energy intake and energy gain was linear and the resulting slope describes the partial efficiency by the sea urchin for energy deposition. The digestible energy requirement for maintenance was calculated to be 32.8 J / g urchin / day and for digestible protein 0.56 mg / g urchin /day. The partial efficiency of utilization for growth was 0.25 and 0.39 for digestible energy and digestible protein respectively.

Using these values together with a suitable growth prediction allow optimization of feeds to contain the proper energy to protein ratio for *Paracentrotus lividus*.

QUANTIFICATION OF QUAHOG PARASITE UNKNOWN (QPX) IN ENVIRONMENTAL SAMPLES

M. Maille Lyons (University of Connecticut), Steven Roberts (Marine Biological Laboratory), Chris Dungan (Maryland Department of Natural Resources), Roxanna Smolowitz (Marine Biological Laboratory), Evan Ward (University of Connecticut)

Quahog Parasite X (QPX) is a pathogen of the bivalve mollusc, *Mercenaria mercenaria*, commonly called a quahog or hard clam. The objective of this study was to develop and validate a quantitative polymerase chain reaction (qPCR) assay that is sensitive and specific for detection of the QPX parasite in experimental and environmental samples. Unique aspects of this qPCR assay development include quantifying the serial dilutions of QPX cells before DNA extraction to calibrate standard curves to cell counts and the targeting of a novel potassium channel gene for amplification. Sensitivity was evaluated with serial dilutions of cultured cells to determine the lowest concentration of DNA that remained detectable in the presence and absence of potential inhibitory environmental

compounds. Based on our results, the qPCR assay can effectively quantify QPX within the range of 1 to several thousand organisms. Specificity was assessed by testing 29 thraustochytrid-like protist strains isolated from oysters (*Crassostrea virginica*, *Crassostrea ariakensis*), soft shell clams (*Mya arenaria*) or quahogs (*Mercenaria mercenaria*) from Virginia, Chesapeake Bay, China, or Oregon. This technique will provide a valuable tool for characterizing QPX abundances in coastal environments and for improving QPX disease diagnoses in clams.

EVALUATION OF ARTIFICIAL OYSTER REEF DESIGN FOR OYSTER HABITAT RESTORATION IN GEORGIA.

Justin Manley (Savannah State University), Randal L. Walker (University of Georgia Marine Extension Service), Alan Power (University of Georgia Marine Extension Service), Dorset Hurley (Sapelo Island National Estuarine Research Reserve), Mathew Gilligan (Savannah State University), Joseph P. Richardson (Savannah State University)

The eastern oyster *Crassostrea virginica* is a common estuarine species along the eastern seaboard of the United States and forms dense intertidal fringing reefs in coastal Georgia. Oyster reef acreage has decreased in Georgia over the last century as a result of commercial harvesting, rapid coastal development, and disease epidemics (*Perkinsus marinus* and *Haplosporidium nelsoni*). Spat abundance is not the limiting factor (settlement rates up to 204,700 m²); rather it is the availability of cultch. Southeastern estuaries are dominated by muddy substrates with oyster reefs forming the only hard substrate. The insufficient return of shell has been detrimental to sustaining oyster populations. The purpose of this study was to determine an optimal cultch material and design for oyster restoration in this environment. Fresh oyster shell, old washed oyster shell, and whelk shell were separately placed into crab traps and plastic mesh bags. Spat collection sticks (PVC tubes embedded with calcium carbonate chips) were arranged in densities of 81 m² and 25 m². Treatments were placed in the Duplin River, Sapelo Island in April 2004. Oyster reef acreage in this area decreased from 8.9 ha in 1891 to 4.3 ha by 2000. Mean annual oyster growth and mortality rates were assessed for each treatment after a one-year period. The collection sticks in the 81 m² treatment performed best (mean annual growth rate: 77.09 mm/year, mortality: 3.467 ± 1.423 %). Structures with higher vertical placement reduce complications associated with high siltation rates and may provide greater surface area for larval attachment.

AGE-SPECIFIC MORTALITY RATES AND CAUSES OF MORTALITY IN NATURAL OYSTER, CRASSOSTREA VIRGINICA, POPULATIONS.

Roger Mann (Virginia Institute of Marine Science), Melissa Southworth (Virginia Institute of Marine Science), Juliana M. Harding (Virginia Institute of Marine Science), Ryan Carnegie (Virginia Institute of Marine Science)

There are very few estimates of natural mortality rates in natural populations of oysters *Crassostrea virginica* (as opposed to abundant estimates from culture and tray type experiments). We offer two methods to estimate natural mortality rates over extended periods and multiple reefs systems for populations in the James River, VA under varying disease challenges. We convert size specific live and box data to age specific demographics, and then generate estimates of age specific mortality from either box ratio (#box / (#box + #live)) or inverse survivor ratios (Live(t) – Live (t+1)/Live (t)). These are presented and compared with respect to oyster population density, prevalence and intensity data for *Perkinsus marinus*, and river flow data as a surrogate for salinity.

YOU CANNOT GET THERE FROM HERE, CAN YOU? AN EXAMINATION OF THE TIME COURSE AND IMPACT OF OYSTER RESTORATION IN CHESAPEAKE BAY

Roger Mann (Virginia Institute of Marine Science), Juliana M. Harding (Virginia Institute of Marine Science), Melissa Southworth (Virginia Institute of Marine Science)

While restoration of the oyster resource of the Chesapeake Bay is supported by extensive research and “on the ground” efforts there has been a reluctance to estimate a realistic time course for achieving both restoration goals and eventual magnitude of impacts on ecological processes, water quality and support of commercial fisheries (noting that these may not be equivalent). Fundamental aspects of oyster biology dictate that such estimates will have similar limitations no matter what species (singular or plural, native or non-native) is considered. We describe the current state of knowledge of stock-recruit relationships, annual replacement ratios, growth rates, age-specific mortality rates in relation to density and disease pressure, and available habitat to provide upper and lower estimates of time to stable terminal carrying capacity (with implications for ecological services and water quality) and potential harvest for commercial purposes.

CONDITION INDICES OF THE EASTERN OYSTER AND REEF CHARACTERIZATION AS RELATED TO WATER QUALITY IN THREE TIDAL CREEKS

Anne L. Markwith (UNC-Wilmington), Martin H. Posey (UNC-Wilmington), Troy D. Alphin (UNC-Wilmington)

The eastern oyster (*Crassostrea virginica*) is an ecologically and commercially important species, providing a series of ecosystem functions (erosion control, filtration, habitat nutrient cycling). This study focuses on the dynamic interaction between oysters and water quality. Through filtration and increased sedimentation oysters have been shown to reduce particulate load in the water column. However, oyster condition and health are also closely linked to water quality conditions, especially total suspended solids (TSS) and sedimentation rates. We expect to see a correlation between the general background water quality, general oyster reef characteristics (density, percent cover, size demography, and rugosity) and measures of oyster condition, such as soft tissue dry weight, volume, and shell characteristics. Data was collected during two sampling periods in three tidal creeks in southeast North Carolina that vary in background water quality characteristics. Sampling occurred during summer (July/ August)2005 and during winter (December 2005/ January 2006). All sampling was conducted in the middle section of the creeks, where tidal regime and salinity were similar among sites. Condition index was performed on fifteen randomly selected oysters in two different size ranges per creek using Hopkins’ formula: $C.I. = (\text{weight of dry tissue})(100) / (\text{internal shell volume})$. Water quality data and information on watershed development was provided by a long term monitoring project. It was hypothesized that the healthier oysters would be found in the creeks with the historically better water quality levels. Preliminary analysis of the data from the creeks however does not support the hypothesis.

CONDITION INDEX OF SURF CLAMS (SPISULA SOLIDISSIMA) IN THE MID-ATLANTIC BIGHT

Rebecca J. Marzec (Haskin Shellfish Research Laboratory, Rutgers University), Yungkul Kim (Haskin Shellfish Research Laboratory, Rutgers University), Eric N. Powell (Haskin Shellfish Research Laboratory, Rutgers University)

Scientists from the Haskin Shellfish Research Laboratory and National Marine Fisheries Service-Northeast Fisheries Science Center conducted a survey of surf clam (*Spisula solidissima*) stocks in an area from northern New Jersey to southern Virginia during June and July of 2004 in order to evaluate the progress of mortality, apparently related to warming of the Mid-Atlantic Bight. One component of the survey was the measurement of condition index at 104 locations from Delmarva to Long Island. The program followed a pilot study in 2002 that suggested that surf clam mortality off Delmarva was likely caused by warmer temperatures decreasing feeding and subsequently leading to starvation. All sampling was conducted aboard the *F/V Lisa Kim*, a commercial clammer. Condition index was highest inshore, with the exception of a few of the most inshore stations, and lowest at the offshore edge of the clam’s range. An estimate of meat weight for a standard 120-mm clam, from site-specific length-weight regressions, revealed that the animals near the center of the inshore-offshore distribution had a greater weight for a given length than those living at the edges of the clam’s range, probably due to the influence of temperature on feeding and growth. Low condition in the extreme inshore locations suggests that warmer temperatures continue to negatively affect surf clam nutrition and indicate the continued susceptibility of clams along the southern and inshore boundary to warming in the Mid-Atlantic Bight.



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FOULING CONTROL FOR OFF-BOTTOM OYSTER CULTURE USING THE ADJUSTABLE LONGLINE SYSTEM

Vanessa Maxwell (Louisiana Sea Grant College Program), John Supan (Louisiana Sea Grant Development Program)

The Eastern oyster *Crassostrea virginica* is an important commercial species in Louisiana. The production of oysters from Louisiana has decreased due to predation, habitat destruction, disease, and the moratorium on leases. Off-bottom culture may be a feasible alternative to increase production, but fouling organisms, such as barnacles and bryozoans, can obstruct mesh openings, reducing growth and survival, and increasing maintenance. The Adjustable Longline System (ALS), commercially used in Australia, allows the oysters to be held at various depths to control culture conditions. This study used the ALS to determine a method of controlling biofouling by measuring 1) growth, 2) survival, and 3) fouling. At the Sea Grant Grand Isle Oyster Hatchery, the ALS was used to test 4 different treatments: a) weekly aerial drying; b) a monthly brine dip; c) daily exposure at low tide; and, d) a no treatment control. Shell height was measured monthly. Fouling was measured monthly by counting organisms on ceramic tiles held on longline cable alongside oyster bags. Total fouling on the oysters was measured by volume at harvest. Oysters reached the legal market size of 76 mm using the ALS within 12 months. Weekly aerial drying had less fouling than either the control or brine dip treatments. The low-tide treatment had the least fouling, but the slowest growth rate. The efficient treatment was weekly aerial drying.

DERMO DISEASE IN STANDARD AND DISEASE-TOLERANT STRAINS OF THE OYSTER (CRASSOSTREA VIRGINICA) IN THE PATUXENT RIVER, MD

Carol B. Mccollough (Maryland DNR, Cooperative Oxford Laboratory, Oxford, MD 21654), Christopher F. Dungan (Maryland DNR, Cooperative Oxford Laboratory, Oxford, MD 21654), George R. Abbe (Morgan State University, St. Leonard, MD 20685)

Disease-tolerant strains of the eastern oyster may be of value in population restoration efforts if they outperform the standard strain in survival and growth. Dermo disease measures in two disease-tolerant strains, DEBY and CROSBreed, were compared to those in standard oysters deployed along a salinity gradient in the Patuxent River. In September 2003 tolerant strains were deployed at a size of 3-4 mm; standards were deployed at 16 mm. Whole body burden dermo disease assays were conducted monthly from the time of deployment. Salinities were depressed in 2003 due to above average freshwater inflows. During 2004 and 2005 flows in the Patuxent River returned to normal ranges. *Perkinsus* sp. infections were detected in all strains at all sites during 2004. Typical seasonal patterns in prevalence developed during both 2004 and 2005. Mean sample body burdens from all strains rarely exceeded 30 parasites/gm tissue wet weight, and were typically less than 1, except at the downriver site in September 2005 where body burdens in standards and DEBYs reached and exceeded 10² parasites/g. DEBYs exhibited delayed or depressed prevalence peaks compared to CROSBreeds and standards at all sites during the second year; however, prevalences in this strain were not consistently lower than both the others, except at the mid-river site. At this time, strong differences in disease progression between strains are not apparent, but may develop during the third year as these experimental oysters endure further disease pressure as they grow to market size.

RESEARCH PROGRESS ON WHITE ABALONE (HALIOTIS SORENSENI)

Thomas B. McCormick (Channel Islands Marine Resource Institute)

White abalone (*Haliotis sorenseni*), a large gastropod mollusc in the family Haliotidae, was once found in rocky subtidal ocean waters off the west coast of North America from Point Conception in California, south to Punta Abreojos, Baja California, Mexico. Long recruitment intervals, site fidelity, fishing, poaching, disease, and habitat change have contributed to its decline. Surveys of historic habitat in the 1990s indicated that population densities had dropped to less than 0.1% of the estimated pre-exploitation population size. A status review led to a 2001 listing of the white abalone as the first endangered marine invertebrate under the Endangered Species Act of 1976 (66 FR 29053). A cooperative program of research is now providing information many aspects of white abalone

biology. Large-scale cultivation of hatchery-raised white abalone is providing an opportunity to study all life stages of this invertebrate. This presentation will provide a summary of research on age at first sexual maturation, optimal cultivation temperature, density, and diet. Behavioral studies suggest that white abalone are capable of rafting on macroalgae, and may have far greater dispersal capabilities than previously thought.

USE OF A BIOENERGETICS MODEL TO INVESTIGATE THE HABITAT USE AND BEHAVIOR OF A NONINDIGENOUS CRAB, *CARCINUS MAENAS*

P. Sean McDonald (University of Washington, SAFS), Kirstin K. Holsman (University of Washington, SAFS), David A. Beauchamp (University of Washington, SAFS), Brett R. Dumbauld (USDA, Agricultural Research Service), David A. Armstrong (University of Washington, SAFS)

We developed an individual-based bioenergetics model to investigate habitat use and migration behavior of non-indigenous European green crab (*Carcinus maenas*) in Willapa Bay, Washington, USA. The model was parameterized using existing studies and applied to four scenarios of habitat use and behavior during a 214-day simulation period (April – October), including crabs occupying (1) sublittoral habitat, (2) sublittoral habitat but undertaking intertidal migrations (3) mid-littoral habitat, and (4) high littoral habitat. Monthly trapping was done along an intertidal gradient to determine the distribution of crabs for the same time interval as the simulation period, and we compared model results to the observed pattern. Model estimates suggest no intrinsic energetic incentive for crabs to occupy littoral habitats since metabolic costs were ~ 6% higher for these individuals than their sublittoral counterparts. Crabs in the littoral simulations were also less efficient at converting consumed energy into growth than sublittoral crabs. Yet trapping revealed abundant *C. maenas* in mid-littoral habitats of Willapa Bay and no evidence of resident sublittoral populations. The discrepancy intimates the significance of interspecific interactions that are not incorporated into the model but nonetheless increase metabolic demand. Agonistic encounters with native Dungeness crabs (*Cancer magister*) may be chief among these added costs, and *C. maenas* may largely avoid interactions by remaining in littoral habitats neglected by native crabs. We suggest that *C. maenas* in Willapa Bay occupy tidal elevations that minimize encounters, and thus metabolic costs, while simultaneously maximizing submersion time and foraging opportunities.

DISTRIBUTION AND QUANTIFICATION OF MULTIXENOBIOTIC RESISTANCE PROTEINS IN THE SEA URCHIN, *LYTECHINUS ANAMESUS*

Nature A. McGinn (University of California - Davis, Bodega Marine Laboratory), Gary N. Cherr (University of California - Davis, Bodega Marine Laboratory)

Multixenobiotic resistance (MXR) is an efflux mechanism that uses transport proteins to remove foreign chemicals and waste products from cells. A taxonomically diverse group of marine organisms express MXR transport proteins and in many larval forms, MXR appears to be a first line of defense against natural toxins and contaminants. Research has shown that marine species express transport proteins similar to mammalian P-glycoprotein (P-gp) and multidrug resistance-associated proteins (MRPs). MXR proteins are found in molluscan gills where MXR also appears to play a protective role. However, few studies have examined other adult tissues in marine species for expression of MXR proteins. In mammals, homologous multidrug resistance (MDR) proteins occur in tissues with important protective functions including the blood brain barrier and placenta. In this study we are using the sea urchin, *Lytechinus anamesus*, to investigate the expression and potential function of MXR transport proteins in an adult model marine organism. Preliminary Western blot analysis with a monoclonal antibody indicates expression of P-gp in adult sea urchin tissues including gonads and coelomocytes. We are surveying adult tissues including gonads, gut, tube feet, and coelomocytes as well as major developmental stages for expression of P-gp and MRPs. Study of the larval stages will help elucidate potential transitions in expression and function of the MXR proteins from developing to adult sea urchins. Results from Western blot analysis and immunohistochemistry with custom sea urchin antibodies will be presented. Protein expression data can serve as a baseline for future toxicological research with sea urchins.

THE EFFECTS OF AMMONIA ON THE DEVELOPMENT, SURVIVAL AND METAMORPHIC SUCCESS OF STROMBUS GIGAS VELIGERS

Melissa Mcintyre (Florida Institute of Technology), Megan Davis (Harbor Branch Oceanographic Institution), Amber Shawl (Harbor Branch Oceanographic Institution)

The Florida Keys queen conch population was a vital fishery, but heavy fishing pressure forced the closure of the commercial fishery in 1976. Since the closure, the recovery of the population has been minimal due to low recruitment of veligers. The queen conch larval stage lasts 2-6 weeks and during this time veliger survival may be affected by contaminants. Ammonia enters the water through sewage leaching and agricultural runoff. Ammonia is toxic to coral planulae, but toxicity of NH₃ to queen conch larvae has not been established.

This study examined the effects of NH₃ on new hatch, 5 days old, 10 days old, and competent veligers. The treatments tested were artificial seawater; natural seawater; and 1, 10, 100 and 1000 µM of ammonia. Development, mortality and an LC₅₀ was determined for each larval stage. Newly hatched veligers exposed to ammonia had a 15-30% increase in mortality over the control after 96 hours. Five day old veligers had 100% mortality in the NH₃ treatments at 96 hours and an LC₅₀ of 0.8 µM. Ten day old and competent veligers were robust and able to withstand exposure to levels below 1000 µM, with LC₅₀'s of 429 and 576 µM, respectively. Metamorphosis was observed in all treatments, excluding 1000 µM; with natural seawater having the greatest metamorphic success. This study reveals that exposure to NH₃ can compromise the development, survival, and metamorphic success of *Strombus gigas*. The findings of this study will assist in understanding the role ammonia has on recruitment of veligers in the Florida Keys.

COMPARISON OF CRASSOSTREA ARIAKENSIS AND C. VIRGINICA IN THE DISCHARGE AREA OF A NUCLEAR POWER PLANT IN CENTRAL CHESAPEAKE BAY

Richard I. Mclean (Maryland Department of Natural Resources), George R. Abbe (Morgan State University)

The potential for introduction of the non-native *Crassostrea ariakensis* into Chesapeake Bay prompted a 5-mo comparison study with *C. virginica* conducted near the Calvert Cliffs Nuclear Power Plant in Maryland, with the primary goal being a comparison of uptake rates of radionuclides released by the plant. The study began with approximately 200 market size triploid *C. ariakensis* (1-yr old) and 200 diploid *C. virginica* (3-yr old) held in secure stainless steel cages 0.5 m off bottom. Half were deployed from July to September 2004, and half from July to December. Although quantities of radionuclides released by the CCNPP were insufficient to yield detectable concentrations in either species, other data were obtained.

From July to December *C. ariakensis* increased its initial shell length by nearly 20% compared to 9% for *C. virginica*. Although mean shell sizes of both species were similar, meat weights and condition indices for *C. ariakensis* were significantly greater ($p < 0.001$) than for *C. virginica*. *C. ariakensis* had lower overall mortality even though nine were lost to blue crab predation during the first exposure period. After 5 mo of exposure, prevalence of dermo disease in *C. virginica* was 65% with a relative intensity of 1.50 on a 0-7 scale; *C. ariakensis* showed no sign of infection.

Although the non-native *C. ariakensis* may have much to offer with high meat yield and disease resistance, rapid growth of triploids results in very thin shells, which may make them more susceptible to blue crab predation than the thicker-shelled *C. virginica*.

GENOMIC ANALYSIS OF GROWTH IN LARVAE OF CRASSOSTREA GIGAS

Eli Meyer (University of Southern California), Dennis Hedgecock (University of Southern California), Donal T. Manahan (University of Southern California)

Molecular biological analysis has provided important insights into the processes of animal development. However, data on the molecular biological bases of growth are more scant. In particular, the molecular biological bases of hybrid vigor have long remained unresolved despite nearly a century of research and enormous economic importance. We approached this question by comparing global gene expression patterns in slow- and fast-growing larval families of the Pacific oyster *Crassostrea gigas*. These different families of larvae were produced through factorial crosses between inbred lines. A total of 4.5 million cDNAs were cloned from 6-day-old larvae and analyzed by “Massively Parallel Signature Sequencing” (Solexa, Inc.). This analysis resulted in quantitative expression profiles and partial sequence data for each of 52,825 unique cDNAs present. Of these, a small subset of 188 genes were found to be differentially expressed in fast-growing hybrid larvae. By comparison with GenBank, 51 of these cloned genes showed significant similarity with DNA sequences for which a function is known; 23 of which represent ribosomal proteins. We found that 40% of these ribosomal proteins were up-regulated and the other 60% down-regulated in fast-growing larvae. These data demonstrate the feasibility of functional genomic approaches and reveal that regulation of protein synthesis machinery is involved in the molecular mechanisms responsible for hybrid vigor. This approach has resulted in the generation of a set of gene probes and possible “markers” that will likely prove useful as early predictors of rapid growth potential in oysters.

THE USE OF MOLECULAR MARKERS TO ASSESS NATIVE OYSTER RESTORATION EFFORTS

Coren A. Milbury (University of Delaware, College of Marine Studies), Coren A. Milbury (University of Delaware, College of Marine Studies)

Restoration efforts are becoming essential in managing many of our ecological resources. Monitoring the efficacy of these programs is essential for evaluating their cost-effectiveness and testing alternative restoration techniques. A majority of oyster restoration efforts use hatchery-produced strains derived from a native stock; however, identifying seed derived from the native population is difficult and requires a high degree of specificity and accuracy. Recent advances in genetic techniques provide high-throughput cost-effective methods for discriminating outplanted seed and their progeny from natives. Our approach is based on shifts in mitochondrial haplotype frequencies occurring during the course of hatchery propagation. Typical natural populations of oysters possess a single major haplotype and an abundance of rare haplotypes. As a result of population bottlenecks during propagation, hatchery seed will often exhibit elevated frequencies of one or more haplotypes that are rare in the source population, allowing discrimination between hatchery seed and natives. Using high-throughput screening techniques, we have identified mitochondrial single nucleotide polymorphisms (mtSNPs) that distinguish hatchery seed planted in the Chesapeake Bay. These markers will be used to determine if outplanted oysters survived and contributed to local recruitment. Even when enhancement stocks are derived from local broodstocks, high-throughput molecular genotyping techniques can allow effective monitoring of enhancement efforts.

DIGESTIVE ENZYME ACTIVITY OVER ONTOGENY IN BAY SCALLOPS, ARGOPECTEN IRRADIANS, AND SEA SCALLOPS, PLACOPECTEN MAGELLANICUS

Lisa M. Milke (National Research Council, Institute for Marine Biosciences), V. Monica Bricelj (National Research Council, Institute for Marine Biosciences), Neil W. Ross (National Research Council, Institute for Marine Biosciences)

Although stage- and species-specific differences in dietary requirements have been demonstrated for bivalves, the mechanisms responsible for differential diet performance remain largely unexplained. While biochemical composition is central to assessing the nutritional value of a diet, an animal’s digestive and assimilation capacity are equally important. Digestive capacity of sea and bay scallops may differ 1) over ontogeny associated with morphological changes, and 2) between species, resulting from adaptation to different environments and food supply. To address this, scallop postlarvae [initial shell height (SH) ~250 μ m] were reared on a mixed algal diet consisting of one diatom and one flagellate until 4-5 mm SH, and were sampled at intervals coinciding with major morphological changes. Colorimetric assays were conducted to measure the specific activity of carbohydrases (laminarinase, cellulase and alpha-amylase), proteases (azocasein and cathepsin B) and lipase (esterase) in scallop

tissues. While preliminary results show no pattern in protease or lipase activity either between species or over development, clear differences in carbohydrase activities were observed. Sea scallops exhibited higher laminarinase activity, but lower levels of alpha-amylase and cellulase than bay scallops, with the most pronounced changes in enzymatic activity occurring prior to attaining ~1.2 mm SH. However, laminarinase activity was significantly higher than activities of either alpha-amylase or cellulase in both scallop species. Our findings have implications for developing targeted diets for commercial hatcheries and increasing our understanding of bivalve-seston interactions in the field.

NATIVE FLORIDA CRUSTACEAN PREDATORS' PREFERENCES REGARDING THE NON-INDIGENOUS GREEN MUSSEL, *PERNA VIRIDIS*

Emily Mitchem (University of Florida), Jonathan S. Fajans (Florida Institute of Oceanography), Dr. Shirley M. Baker (University of Florida)

The green mussel, *Perna viridis*, a native of the Indo-Pacific, was first reported in Tampa Bay in 1999 (Benson et al. 2001; Ingrao et al. 2001). In order to gain a better view of what this invasion might mean for Florida's waters and its native species, this experiment posed the following two-fold question: (1) Will native crustacean predators prey on *P. viridis*? and (2) Will these predators prefer native bivalves over *P. viridis*? Due to the high commercial value of *Panulirus argus*, the Caribbean spiny lobster, and *Callinectes sapidus*, the blue crab, these crustaceans made suitable predator subjects. The American oyster, *Crassostrea virginica*, and hard clam, *Mercenaria mercenaria*, were chosen as competing prey because these bivalves are commercially important within Florida. Crustaceans and bivalves were placed in a recirculating system with eight 624 liter tanks. Filtration systems included a protein skimmer and fluidized bed, wet-dry filter, and ultraviolet sterilization. Trials ran for fourteen days. *Panulirus argus*, consumed one *C. virginica*, and seventeen *M. mercenaria*. *Callinectes sapidus*, consumed fifteen *P. viridis*, seven *C. virginica*, and seven *M. mercenaria*. A one-way analysis of variance was used to analyze data. A p-value less than 0.05 was considered statistically significant. For the *P. argus* experiment, $p=0.0219$, showing that *P. argus* data were significant. After this, a Tukey Test was performed. *Callinectes sapidus* data were not statistically significant because $p=0.2067$, although *C. sapidus* consumed twice as many *P. viridis* than either *M. mercenaria* or *C. virginica*.

SABELLID POLYCHAETE INFESTATIONS OF FARMED CALIFORNIA ABALONE

James D. Moore (California Department of Fish and Game), Christy I. Juhasz (UC Davis Bodega Marine Laboratory), Thea T. Robbins (California Department of Fish and Game)

The South African sabellid polychaete *Terebrasabella heterouncinata* lives in shells of abalone. Accidentally introduced to California during the 1980s, it was spread via abalone shipments to abalone culture and display facilities throughout the state. Eradication efforts have been largely successful although concern remains about whether it has become established in intertidal gastropod populations adjacent to previously-infested facilities. We found that sabellid transmission can occur among native turban snails *Tegula funebris*, corroborating the potential risk of sabellid infestations in native, non-haliotid gastropod populations. Nevertheless, a state-wide survey of turban snails and limpets at 21 exposed or potentially exposed locations found no sabellids, suggesting that the pest has not become established in California.

Freshwater treatment is commonly used to sanitize holding units, hands and equipment at farms and display facilities, but the amount of freshwater required to kill sabellids and their larvae was not well defined. We found that sabellid transmission occurred from infested abalone shells that had been immersed in freshwater for up to 8 hours. Additionally, motile sabellid larvae survived up to 32 seconds of immersion in freshwater. We produced an informational video to provide facility staff with recommended practices to achieve eradication and minimize chances of re-infestation. Supported in part by the University of California Integrated Pest Management Program and the Marine Region, California Department of Fish and Game.

COMPARISON OF BACTERIA UPTAKE AND DEPURATION BETWEEN *CRASSOSTREA ARIAKENSIS* AND *CRASSOSTREA VIRGINICA*

James A. Morris Jr. (NOAA), Tanya J. Bean (University of North Carolina), Rachel T. Noble (University of North Carolina), Patricia K. Fowler (NC Department of Environment and Natural Resources)

Considerations to introduce the Suminoe or Asian oyster *Crassostrea ariakensis* along the East Coast have raised many questions regarding ecology, economics, and human health. To date, research has focused primarily on the ecological and socioeconomic implications of this initiative, yet few studies have assessed its potential impact on public health. Our work compares the rates of bioaccumulation and depuration of indicator organisms (such as *E. coli*) and *Vibrio sp.* between *Crassostrea virginica* and *Crassostrea ariakensis* in the laboratory. Preliminary results suggest that the rates of bioaccumulation of *E. coli* in *Crassostrea ariakensis* were significantly lower than those for *Crassostrea virginica* and that depuration was variable between the two species. This research provides coastal managers with insight into *Crassostrea ariakensis* response to bacteria, an important consideration for determining appropriate management strategies for this species. Further field-based studies will be necessary to elucidate the mechanisms responsible for the differences in rates of bioaccumulation and depuration.

SEANEST – A COMMERCIAL SYSTEM FOR LARGE SCALE CULTURE OF BENTHIC SPECIES

Vidar Mortensen (Praktisk Teknologi AS, Norway)

Sea based culture of benthic invertebrates is very labour intensive. This is especially true for species like sea urchin, which require frequent feeding. In addition, traditional equipment like oyster trays fails to accommodate the biological demands of sea urchins. The SeaNest system has been developed to solve these problems, and to provide the farmer with a modern workplace. Time consuming physical labour is avoided through automatic handling of the holding boxes, and the operator has an ergonomically good position while running the system. The system is designed to meet the biological requirements of sea urchins, with respect to surface area, water exchange, waste removal and short time of air exposure. Further developments towards automatic feeding, cleaning and tracking of holding boxes will provide additional benefits for the farmer.

The systems main concepts and technical features will be presented. Prototypes of the system are in use at a commercial sea urchin farm in Norway. The experiences from this farm will be discussed both with regards to sea urchin farming in general, and the function of the SeaNest system in particular.

(The SeaNest system is patented, with further patents pending).

OBSERVATIONS OF ASIAN PERKINSUS SPECIES AND PERKINSUS MARINUS IN THE SUMINOE OYSTER, *CRASSOSTREA ARIAKENSIS*

Jessica A. Moss (VIMS, The College of William and Mary), Kimberly S. Reece* (VIMS, The College of William and Mary)

Decline of *Crassostrea virginica* populations has led to an interest in using the non-native oyster species, *Crassostrea ariakensis*, for aquaculture development, fishery resource enhancement and habitat restoration in the Chesapeake Bay region. Previous field studies comparing the performance of *C. ariakensis* and *C. virginica* in the region suggested that the non-native oyster was substantially more tolerant of the endemic protozoan parasite, *Perkinsus marinus*. We conducted a disease survey of *C. ariakensis* in China, Japan and Korea using molecular diagnostics. PCR-based assays revealed two *Perkinsus* species in Asian Suminoe oyster populations that currently are not found in US waters; *Perkinsus olseni* and, based on DNA sequence data, an undescribed *Perkinsus* species. We will report on progress towards molecular characterization of this undescribed *Perkinsus* species. Quarantine protocols for *C. ariakensis* brought to the US from Asia should limit introduction of exotic pathogens, however, it is important to understand the impact that Asian *C. ariakensis* pathogens could have on local bivalve species in case of accidental introduction via the host or through an indirect source such as ballast water, in which case the non-native

oyster could act as a pathogen reservoir. We will report results of challenge experiments being conducted with Asian *C. ariakensis* pathogens to examine the impacts on the local Chesapeake Bay bivalve species, *C. virginica* and *Mercenaria mercenaria*, as well as on *C. ariakensis*. During these experiments, we unexpectedly observed the acquisition of moderate and heavy intensity infections of *P. marinus* in *C. ariakensis* oysters being maintained in the laboratory.

GENOMIC EVIDENCE FOR DIVERGENT SELECTION IN EASTERN OYSTER POPULATIONS ACROSS AN ECOTONE

Maria Murray (University of Maryland), Matthew P. Hare (University of Maryland)

The eastern oyster, *Crassostrea virginica*, is broadly distributed along the western North Atlantic coastline. Inside this range, a steep latitudinal temperature gradient and a transition in species composition from temperate to subtropical communities generate an ecotone along eastern Florida. Despite evidence for homogenizing gene flow within large continental regions, presumably mediated by a three-week planktonic larval stage, oyster populations display a sharp genetic cline in eastern Florida. We are interested in discovering the abiotic and biotic mechanisms by which a high gene flow species maintains genetic diversity along the ecotone. Population genomics approaches offer effective ways to statistically test for genetic evidence of divergent selection in this non-model species. We report genomic evidence for the role of selection across the ecotone using 215 amplified fragment length polymorphism loci sampled from two populations experiencing the environmental extremes. Simulations of neutral population divergence provided a statistical framework for testing whether differentiation at some loci is greater than expected by genetic drift. For a small portion (2-7%) of loci, allele frequency differentiation was high enough across the ecotone to reject the neutral drift model ($p < 0.01$). Corroborating this inference with additional population sampling, several of the nonneutral AFLP loci show clinal variation concordant with previously described loci. Also, one AFLP locus was converted to a codominant marker and still shows a significantly nonneutral pattern of differentiation. Thus, we infer that divergent selection along the Atlantic Florida ecotone helps maintain genetic differences between oyster populations in the Atlantic and Gulf of Mexico.

EASTERN OYSTER AND HARD CLAM CULTURE FOR THE REDUCTION OF RAS EFFLUENT NUTRIENT COMPOSITION

Amanda Myers (University of North Carolina Wilmington), Wade Watanabe (University of North Carolina Wilmington), Ami E. Wilbur (University of North Carolina Wilmington)

Closed recirculating aquaculture systems (RAS) are a technological innovation that reduces the amount of water needed for culture by treating and reusing >90% of the total water volume daily. Such systems consequently produce substantially smaller volumes of effluent, but this smaller volume is substantially more enriched in nutrients than effluent from flow-through or open systems. For RAS to emerge as a viable culture strategy, an economical and efficient method must be developed to reduce effluent nutrient concentrations before this effluent is discharged. As part of a larger project evaluating biofilter effects on RAS effluent nutrient composition, this study focused on the effect of bivalve culture on the composition of effluent from a southern flounder RAS. This system produces 1270L/day of enriched effluent, with nutrient and suspended solids concentrations 20-100x that of ambient levels. This effluent was introduced with seawater to an upweller system stocked with bivalves at two densities for 4-6 weeks. Oysters (*Crassostrea virginica*, average shell height = 63.38 ± 0.86) and clams (*Mercenaria mercenaria*, average shell height = 16.65 ± 0.48) were used in separate experiments. Effluent nutrient composition (total nitrogen, total phosphorus, and total suspended solids) was analyzed from samples (collected twice weekly) taken upstream and downstream of each upweller. Bivalve growth was quantified by measurement of shell height at the start and end of each trial. Analysis of preliminary data suggests that both oysters and clams exhibited growth over the course of the experiments. Water samples are currently being processed to determine the impact of bivalve activity on effluent nutrient composition.

STATUS AND TRENDS OF BLACK ABALONE (HALIOTIS CRACHERODII): A PRELIMINARY APPROACH TO MODELING POPULATION VIABILITY



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Melissa Neuman (National Marine Fisheries Service), John Butler (National Marine Fisheries Service), Brian Tissot (Washington State University Vancouver), Daniel Goodman (Montana State University)

Black abalone, *Haliotis cracherodii*, was added to the National Marine Fisheries Service (NMFS) species of concern list in 1999 because of documented large declines, known threats and uncertainty regarding the future course of those threats. Although efforts have been made to protect surviving populations in California, USA through closure of the commercial fishery in 1993, the species continues to decline and a > 90% reduction in abundance has been observed throughout > 50% of the species range since the late 1980s. Heavy fishing pressure and disease have contributed to the observed decline. Expanding coastal development may increase human traffic in the intertidal and may intensify enforcement problems. Pollution, warming temperatures, and biological interactions with competitors/predators may also threaten remaining populations. NMFS must determine whether the rate and extent of black abalone population decline and the severity of risk are high enough to warrant a listing under the United States Endangered Species Act. In order to do this a variety of tools are employed using the best available data to predict the probability distribution of long-term population trends. A population viability model is one tool that is being developed to assess species vulnerability through the analysis of the interplay of specific demographic parameters over a spatial array of habitat suitability and other possible forcing factors. Our scientific challenge is to identify important gaps in our understanding of this species' ecology that will allow us to determine what data needs to be collected for improving the estimates of the most influential parameters.

THE INFLUENCE OF EASTERN OYSTERS ON ECOLOGICAL PROCESSES IN CHESAPEAKE BAY:
INSIGHTS FROM RECENT MODELING STUDIES.

R.I.E. Newell (Horn Point Laboratory, University of Maryland)

Results from mathematical models will be reviewed to examine how changes in eastern oyster abundance may affect benthic-pelagic coupling in Chesapeake Bay. All models indicate that at current abundances of ~1 adult oyster m⁻² on historical oyster bottom, oysters exert negligible influence on water quality. At abundances of ~10 oysters m⁻², equivalent to the goal of the Chesapeake Bay Agreement of a 10-fold increase by 2010, models indicate that during warmer months oysters grazing on phytoplankton reduce turbidity to a level that significantly increases light availability for benthic plants. In cooler months, including during the spring phytoplankton bloom, oysters do not feed sufficiently actively to exert top-down grazer control. Biodeposition leads to appreciable removal of nitrogen through denitrification and burial. Resulting reduced phytoplankton biomass decreases microbial degradation of carbon beneath the pycnocline and increases oxygen concentration in bottom water. At ~100 oysters m⁻², which is the estimated pre-colonial density, models indicate that oysters can exert a profound influence on water quality. Even though such ecosystem services are extremely valuable, the challenge is how to restore oysters in the face of relentless harvest pressure, disease epizootics, and the siltation of the cultch essential for larval settlement. Closure of the public fishery and the development of oyster aquaculture would help increase oyster abundances. An absence of leadership is preventing these necessary changes from occurring; indeed, management decisions often exacerbate the problem, such as the recent opening of Maryland oyster bars to the once illegal practice of oyster dredging.

CHANGES IN SHELL STRENGTH OF CRASSOSTREA VIRGINICA AND CRASSOSTREA ARIAKENSIS IN
RESPONSE TO CRAB PREDATORS FROM CHESAPEAKE BAY.

Roger I. E. Newell (Horn Point Laboratory, UMCES), Victor S. Kennedy (Horn Point Laboratory, UMCES), Kristi S. Shaw (Horn Point Laboratory, UMCES)

If *C. ariakensis* were to be introduced to Chesapeake Bay it is unknown if its abundance would be controlled by the same predators that affect *C. virginica* populations. In replicated laboratory studies, we compared the relative susceptibility of juvenile diploids (shell height < 25 mm) of both oyster species to five species of potential crab predators. We tested two species of mesohaline mud crab, *Rhithropanopeus harrisi* [carapace width 6-16 mm] and *Eurypanopeus demissus* [6-18 mm]), two species of polyhaline mud crab *Panopeus herbstii* [9-29 mm] and *Dyspanopeus sayi* [8-20 mm], and the euryhaline blue crab, *Callinectes sapidus* [35-65 mm]. We found that all four species of mud crab and the blue crab significantly ($P < 0.05$) preferentially consumed *C. ariakensis* compared to *C.*

virginica. We hypothesized that this differential predation may stem from differences in shell strength between the two species of oysters. Using an Instron load compression instrument we found that the strength of the upper valve of *C. ariakensis* was ~50% lower than for comparably sized *C. virginica*. Oysters of both species grown for 54 d in the presence of (but protected from) blue crabs exhibited different responses. *C. ariakensis* grew denser shells (total weight/shell area) that approximately doubled their compression strength compared to same species control oysters. The shell strength of *C. virginica* also approximately doubled, but these stronger shells had a significantly lower density than their controls. We conclude that both species of oysters respond morphologically to the presence of predators, although the response differs markedly between species.

DEVELOPMENT OF LANDSCAPE MODELS FOR PROTECTION AND RESTORATION OF FRESHWATER MUSSELS IN LARGE RIVERS

Teresa J. Newton (USGS Upper Midwest Environmental Sciences Center), Michelle R. Bartsch (USGS Upper Midwest Environmental Sciences Center), Jennie S. Sauer (USGS Upper Midwest Environmental Sciences Center), Jeff J. Steuer (USGS Wisconsin Water Science Center), Steve J. Zigler (USGS Upper Midwest Environmental Sciences Center)

We assessed whether the spatial distribution of mussels could be predicted from physical, hydraulic, and biologic variables in a large floodplain river. Analyses of data from mussel beds at small (0.4 km) and moderate (6 km) spatial scales indicated that computed hydraulic variables (e.g., shear stress, Froude number) were more predictive than simple, measured variables (e.g., depth, velocity, substrate particle size). Similarly, classification tree models of mussel presence-absence in a 38-km reach of the river, which had a prediction success of ~75%, were largely driven by shear stress and substrate stability, but interactions with simple physical variables (e.g., slope) were also important. Moreover, discharge-specific models suggested that episodic events such as droughts and floods were more important in structuring mussel distributions than conditions during average flows. Because mussel species differ in shell features that may influence displacement during high discharge events, we evaluated if sculptured, thick-shelled species occupied areas with higher hydraulic stresses, compared to non-sculptured, thin-shelled species. Classification tree models predicted the presence of all mussels, regardless of shell morphology, in areas with low to moderate hydraulic stresses under low and high flow conditions. Overall, our studies suggest that mussels are influenced by a complex interaction of biotic and abiotic factors acting at various spatial scales, but that certain hydraulic variables can improve our ability to predict their spatial distribution in large rivers.

UNIONID FEEDING STRATEGIES

S. J. Nichols (U. S. Geological Survey-Great Lakes Science Center)

The continued decline of many unionid communities throughout North America has been attributed to many factors ranging from pollution to exotic species. However, one main factor, food supplies, is rarely discussed. This raises a question: are populations declining in some locations because critical food supplies are no longer present? A review of the current research on unionid feeding highlights our limited knowledge about dietary requirements. Feeding studies on wild populations have been limited because of the difficulties in separating assimilation from ingestion in filter-feeding organisms. Recent technological advances such as stable isotopes and C¹⁴ enrichment have provided some information on food selectivity and have shown that unionids feed on a wide range of items including algae, dissolved organic molecules and bacteria. But these studies have not detected any food resource partitioning between sympatric genera or species. All species, regardless of shell length, type of gill cirri, siphon structure, or physical location in the substrate, appear to feed on particles <29 microns and can obtain food from either planktonic or benthic sources. However, such apparent omnivory does not translate into easily-maintained adults under captive conditions. Without access to natural food supplies, adult unionids rarely thrive. Dietary supplements such as used for marine bivalves do not support adult survival, though larvae can be reared using marine algae. Apparent omnivory is obviously misleading regarding actual dietary needs. In-depth surveys of potential food resources in waters with healthy unionid populations are critically needed to understand the relationship between food resources, unionid feeding strategies, and long-term survival.

PHYSIOLOGICAL BASES OF GENETICALLY DETERMINED VARIATION IN GROWTH OF BIVALVE LARVAE (CRASSOSTREA GIGAS)

Douglas A. Pace (University of Southern California), Adam G. Marsh (University of Delaware), Patrick Leong (University of Southern California), Allison Green (University of Southern California), Dennis Hedgecock (University of Southern California), Donal T. Manahan (University of Southern California)

Variation in growth rates has been well documented in larval stages of bivalve molluscs. The endogenous processes that might contribute to individual-based variations in growth rates are not well understood. In this study we used inbred lines of the Pacific oyster, *Crassostrea gigas*, in a series of factorial crosses to produce families of larvae with different growth rates that were genetically set, even under identical environmental conditions of food and temperature. Growth rates of these larval families varied over a 5-fold range, from 3.4 to 17.6 mm×day⁻¹. A suite of integrated measurements were applied to study growth variation in larvae, including: size, biochemical compositions, rates of acquisition of both dissolved and particulate nutrients, absorption efficiencies, metabolic rates, and enzyme activities. Fast-growing larvae had similar size-specific metabolic rates and capacities to slow-growing larvae, but the fast-growing larvae had significantly higher size-specific feeding rates. Also evident was the complex regulation of enzymatic activities responsible for ion transport (a metabolically expensive process in animals). Our results show that while feeding rates were important in explaining a portion of the growth differences, the regulation and internal allocation of metabolic energy played a large role in genetically-set growth rate variation. The larvae studied here clearly exhibited the phenomenon of hybrid vigor (“heterosis□?”). Our results will be discussed in the context of understanding the physiological bases of growth heterosis.

INVASION OF CRASSOSTREA GIGAS IN MARINE RESERVES: LOCAL REPRODUCTION OR DISPERSAL FROM ELSEWHERE?

Dianna K Padilla (Stony Brook University), Terrie Klinger (University of Washington)

The Pacific oyster, *Crassostrea gigas*, has been an important aquaculture species in the Pacific Northwest for > 75 years. Because this species requires waters 19-20°C for spawning, it has been considered a safe introduction, without the possibility of escape due to local reproduction limits. However, *C. gigas* has been invading rocky shores in the San Juan Archipelago, Washington for almost a decade, and is more abundant within than outside marine reserves. This recent invasion could be due to: 1) changes in temperature such that spawning can be induced, 2) thermal loading during low tide (mid-day) that allows oysters to spawn even though the water is cold, 3) evolution for spawning at lower temperatures, or 4) spawning or larval release elsewhere and dispersal into marine reserves and adjacent shores. We tested among these hypotheses by monitoring local temperatures and experiments to determine if oysters collected from the field will spawn when held at ambient water temperatures or at ambient water temperatures when exposed to a tidal cycle. We also tested whether larvae produced by local *C. gigas* were capable of development in the cold waters typical of the San Juans. These oysters do not appear able to spawn at temperatures < 17-18°C, and larval development even at 15°C is slowed > 3 times from development at 20°C. Even in quite bays, temperatures are not warm enough to induce spawning. These data suggest that larval production elsewhere must be responsible for this invasion.

EVALUATION OF THE ANTIMALARIAL DRUG QUININE AS A POTENTIAL CHEMOTHERAPEUTIC AGENT FOR THE EASTERN OYSTER PARASITE, P. MARINUS

Christina Panko (Florida Gulf Coast University), Aswani Voley PhD (Florida Gulf Coast University), Vincent Encomio PhD (Florida Gulf Coast University), Jose Barreto PhD (jbarreto@fgcu.edu)

Currently no anti-protozoal drug therapy exists in aquaculture to effectively treat or prevent *Perkinsus marinus* infections of the eastern oyster. Development of a chemotherapeutant specific for treating oysters infected with *P. marinus* or preventing disease may be an additional measure to avert physiological strain on aquaculture brood stock before spawning. Given the taxonomic proximity of *P. marinus* to Apicomplexans such as the malarial parasite, *Plasmodium spp*, the antimalarial drug Quinine was screened for drug sensitivity of cultured *P. marinus*. Following an acute challenge with varying concentrations of Quinine HCl, the viability of one strain of cultured meronts isolated from Laguna Madre, Texas oysters was determined using a dye reduction method. After a three hour



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exposure, the highest concentration tested, 50µg/ml, had the most significant effect ($p=0.000$) on parasite viability. Following assay optimization, six geographically distinct *P. marinus* isolates (ranging from Connecticut, New Jersey, Maryland, Virginia, Louisiana, and Texas) were exposed to 50µg/ml Quinine HCl to evaluate differences in susceptibility. The viability of all isolates significantly decreased after Quinine exposure. The Maryland and Virginia strains appeared to be more susceptible to treatment than the other isolates. Bearing in mind that hemocytes are integral component of the host innate immune system, the effect of Quinine on hemocyte viability was determined using a Neutral Red assay. The resulting information indicated the importance of examining the use of Quinine in mitigating *P. marinus* infections under *in vivo* conditions.

**ESTABLISHING BASELINES FOR RECOVERY OF OYSTER (CRASSOSTREA VIRGINICA)
POPULATIONS IN SOUTHEAST FLORIDA**

Melanie L. Parker (Fish & Wildlife Research Institute), William S. Arnold (Fish & Wildlife Research Institute)

Oysters occur in nearly all estuarine and nearshore waters along the Florida coast. Many of these waters have experienced altered patterns of water delivery and quality as a result of water management practices related to the St. John's River and Kissimmee River basins, Lake Okeechobee and the Everglades. Alterations in freshwater flow have reduced or eliminated many oyster reefs and impacted both the timing and extent of oyster reproduction. The Comprehensive Everglades Restoration Program (CERP) is being implemented as a means of reinitiating natural freshwater flow to coastal waters in south Florida. Because of its wide distribution and essential habitat value, the eastern oyster is included in this program as a target species for monitoring and restoration. Changes in oyster distribution and abundance, reproductive development, disease incidence, and juvenile recruitment and growth are being monitored at several sites along the southeast coast of Florida including the St. Lucie estuary, Loxahatchee River, Lake Worth Lagoon and Biscayne Bay. Oyster populations in Tampa Bay, Mosquito Lagoon and Sebastian River are also being monitored as a background for comparison because these are outlier populations not affected by CERP water management practices. In January 2005, study sites were established within each estuary and monthly sampling of recruitment, reproductive development and disease incidence was initiated. Adult surveys were also conducted at each study site to determine oyster distribution and abundance. Preliminary results indicate that the pattern of degradation of oyster populations among estuaries is rather chaotic while the pattern within an estuary is more predictable.

**IMPACTS OF THE TOXIC DINOFLAGELLATE ALEXANDRIUM MONILATUM ON THREE
ECOLOGICALLY IMPORTANT SHELLFISH SPECIES**

Susan E. Pate (North Carolina State University), Joann M. Burkholder (North Carolina State University), Sandra E. Shumway (University of Connecticut)

Little is known regarding interactions between shellfish and *Alexandrium monilatum*, a toxigenic dinoflagellate species that forms blooms mostly in the Gulf of Mexico. Toxic *A. monilatum* has been linked to fish and invertebrate kills, and produces endotoxins with hemolytic and neurotoxic properties. We experimentally assessed responses of three ecologically important shellfish species to *A. monilatum* (toxic strain AMO3, NOAA National Ocean Service, Charleston, SC). In the first set of experiments, grazing studies were conducted with adult and juvenile eastern oysters (*Crassostrea virginica*), northern quahogs (*Mercenaria mercenaria*), and green mussels (*Perna viridis*), which inhabit areas where *A. monilatum* blooms occur. Clearance rates of each shellfish species were depressed when exposed to toxic *A. monilatum* (bloom density of ~550 cells/mL) alone or with nontoxic Instant Algae[®] *Pavlova*, in comparison to clearance rates of control animals that were fed a nontoxic dinoflagellate of similar size, *Alexandrium tamarense* (nontoxic clone CCMP115) and benign cryptophyte algae. Exposure to *A. monilatum* also significantly decreased shellfish valve gape. In the second set of experiments, bioassays were conducted to test survival of larval shellfish (*M. mercenaria*, *C. virginica*) exposed to *A. monilatum* as whole, unconstrained cells, cells held in dialysis tubing, or sonicated cells. Sonicated *A. monilatum* caused a significant decrease in larval survival, in comparison to survival of the control larvae that were tested with nontoxic *A. tamarense*. Overall, these data indicate that *A. monilatum* blooms can adversely affect shellfish survival by reducing clearance rate and valve gape, affecting food intake, and inducing larval mortality.

OBSERVATIONS ON THE REPRODUCTIVE BIOLOGY OF THE HAIRY CRAB HAPALOGASTER MERTENSII, ON KODIAK ISLAND, ALASKA.

Susan A. Payne (AFSC-Kodiak Laboratory), Jan A. Haaga (AFSC-Kodiak Laboratory), Dr. Bradley Stevens (AFSC Kodiak Laboratory)

The reproductive biology of female *Hapalogaster mertensii*, an intertidal lithodid crab, from Kodiak Island, Alaska was observed in both the laboratory and field from 2000-2004.

Larval release, in 2001, 2003, and 2004, occurred from February-June in the laboratory, and was not significantly different between years. Hatch duration in the individual crabs varied significantly between years and pairwise comparisons showed the following significant differences between years: 2001 (32.38 ± 3.37 days) > 2003 (22.62 ± 2.08 days) > 2004 (6.50 ± 3.37 days). The average number of larvae released was 214.75 ± 37.66 in 2001 and 157.38 ± 23.24 in 2003, and was not significantly different.

Molting, mating, and the extrusion of a new clutch of eggs are not contiguous as in other lithodid crabs, but occur over a period of 2-5 months. Laboratory observations show that molting occurs twice in some females between hatching and extrusion. Possible mating behavior was observed twice, and occurred after the first molt, but was not associated with the molt or extrusion of new eggs. Egg extrusion in the lab was only observed in 2003 and occurred between 10 August and 10 November, and in the field in 2002 as early as July. Ovary incubation between the crabs was significantly different ranging from 187 to 244 days (224.67 ± 8.4288 , $P=0.00$).

Lab conditions appeared to mimic field observations for the timing of larval release and egg extrusion despite prolonged captivity of lab animals.

TRIPLOID CRASSOSTREA ARIAKENSIS AND CRASSOSTREA VIRGINICA GROWN AT FOUR SITES IN CHESAPEAKE BAY

Kennedy Paynter (University of Maryland), Donald Meritt (University of Maryland Center for Environmental Science), Stan Allen (Virginia Institute of Marine Science), Jake Goodwin (University of Maryland), Marcy Chen (University of Maryland)

Triploid suminoe oysters, *Crassostrea ariakensis*, and triploid eastern oysters, *Crassostrea virginica*, were produced nearly simultaneously in the hatchery and deployed in cages at four sites in Chesapeake Bay in April 2004. The four sites represented the broad range of salinities present in the Bay. The 'high' salinity site was in the York River near the Virginia Institute of Marine Science (VIMS; mean salinity 19 psu). The Patuxent River site had a mean salinity of 11 psu and the Choptank River site of 9 psu. The 'low' salinity site in the Severn River had a mean salinity of 6 psu. Growth of both species was positively correlated with salinity. As of September 2004, no significant differences in size or disease prevalence existed between species at any site. Dermo, the disease caused by *Perkinsus marinus*, was detected in both species in July but only at the York River site. However, *C. ariakensis* grew more later in the year than did *C. virginica* and appeared to begin growing earlier in the following Spring. Thus, *C. ariakensis* became significantly larger by June 2005. During the summer of 2005, *C. virginica* at the Choptank and York river sites grew more slowly than *C. ariakensis*, presumably due to *P. marinus* infections. Mean shell height of *C. ariakensis* at the York river site in October 2005 (deployed 19 months) was 140 mm compared to 105 mm in *C. virginica*. Physiological differences between the species apparently allow *C. ariakensis* to grow more actively at lower temperatures.

USING PCR TO DETERMINE THE ACCURACY OF MORPHOLOGICAL IDENTIFICATION OF MERCENARIA MERCENARIA LARVAE

Laurie Perino (Stony Brook University, Stony Brook, NY), Dr. Dianna Padilla (Stony Brook University, Stony Brook, NY), Michael Doall (Stony Brook University, Stony Brook, NY)

Identification of bivalve larvae in the field is an important aspect of fisheries management, restoration efforts and early warnings of invasive species. Traditional methods of identifying bivalve larvae are based on morphological characteristics of the larval shell. Problems occur when using this method because the larvae of many different species are very similar in shape and size, especially during the smaller D-stage. Newer molecular methods, including multiplex PCR, have been developed to aid in larval identification. The study presented here discusses the accuracy of morphological identification and provides error rates for misidentified larvae by using PCR to identify individual field collected larvae to species. For larvae suspected to be *Mercenaria mercenaria*, the rate of obtaining a false negative (identifying a larva as another species when it is actually *M. mercenaria*) was very low; however the rate of obtaining a false positive (identifying a larva as *M. mercenaria* when it is actually another species) was quite high. These error rates suggest that morphological characteristics are helpful in determining those larvae that are not the target species, but may lead to overestimations of densities of the target species due to overlaps in size and shape of different species.

ENRICHED ARTEMIA NAUPLII AS DIET FOR RED (PARALITHODES CAMTSCHATICUS) AND BLUE (P. PLATYPUS) KING CRAB LARVAE IN THE LABORATORY

Sara Persselin (NOAA Fisheries)

Newly-hatched unenriched *Artemia* nauplii are often the primary diet in culturing red (*Paralithodes camtschaticus*) and blue (*P. platypus*) king crab larvae in the laboratory. Although this diet allows survival to the first juvenile crab stage (C1), rates are suboptimal. This work reports on studies over the last two years investigating whether diets of enriched *Artemia* nauplii improve survival of red (RKC) and blue (BKC) king crab larvae. In 2003, RKC larvae were fed one of seven diets: (1) newly-hatched unenriched *Artemia*, (2-5) *Artemia* nauplii enriched with the microalga *Rhodomonas salina*, *Isochrysis* sp., *Thalassiosira nordenskioldii*, or *T. aestivalis*, or (6-7) *Artemia* nauplii enriched with a commercial enrichment product, *Isochrysis* paste or Algamac 3050. The treatment that resulted in the highest survival to the non-feeding glaucothoe stage was *T. nordenskioldii* (73%) and the treatment that resulted in the highest survival to C1 was *Isochrysis* paste (63%). Lowest survival to both stages occurred in the newly-hatched nauplii treatments (42% and 30%). Based on these results, BKC larvae were fed one of four diets in 2004: 1) *Artemia* nauplii enriched with the diatom *T. nordenskioldii*, 2) *Artemia* nauplii enriched with frozen *Isochrysis* paste, 3) newly-hatched unenriched *Artemia* with *T. nordenskioldii* added to the culture water and 4) no food. The treatment that resulted in the highest survival through C1 was newly-hatched *Artemia* with *T. nordenskioldii* added to the culture water (91.7%). This diet is superior to either enriched *Artemia* nauplii or unenriched newly-hatched *Artemia* for king crab larvae cultured in the laboratory.

THE EFFECT OF CLIMATE CHANGE ON THE DISTRIBUTION AND GROWTH OF ATLANTIC SURF CLAMS, SPISULA SOLIDISSIMA

Adriana Picariello (The Virginia Institute of Marine Science), Roger Mann (The Virginia Institute of Marine Science), Juliana M. Harding (The Virginia Institute of Marine Science)

In recent years, the general distribution of the Atlantic Surf Clam, *Spisula solidissima*, along the east coast of the USA has undergone a dramatic shift both northward and into deeper water. This observation is based on 20 years of NOAA/NMFS/NEFSC stock assessments, with sampling from 1999 to the present indicating that this distribution shift has occurred. We examined surf clam growth from different locations along the current distribution. These locations included animals from both the southern and northern areas within the distribution as well as both inshore and offshore locations within these areas. Samples included a size range of animals 32 mm to 180 mm in shell length. Surf clam ages were determined by counting growth bands in the chondrophore, using methods developed by the Northeast Fisheries Science Center. We present and compare estimates of age structure and growth from surf clam populations along a latitudinal and bathymetric gradient. Fitted growth curves were compared across and within sites.



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THE OTHER SIDE OF RESTORATION

Donatella Del Piero (Biology dept. Trieste univ.)

The tentative restoration done by the fishermen of shellfish beds (mainly *Chamelea gallina*) in Northern Adriatic seems to be far from optimization. Traces of uncontrolled restocking operations dates back at least to 1990 but it doesn't seem to be decisive for stock enhancement. We have, instead, evidence of overall heavy depletion in stock and low growth rates, so the trade organisation are asking for diminishing the minimum legal size, actually set to 25 mm, till 20 mm, due to lack of legally marketable clams. This is a very old problem in the area and is of course questionable if the lack of response e.g. to fleet reduction and long closure season, may reflect changes in the whole system or is an effect of gear improvement, bad knowledge of population dynamic, physiology etc. that must be known for implementing restoration programs.

TEMPORAL EXPRESSION OF GENETIC LOAD IN TWO FAMILIES OF THE PACIFIC OYSTER
CRASSOSTREA GIGAS

Louis Plough (University of Southern California)

Discovery of a large number of deleterious recessive mutations (genetic load) in the Pacific oyster (Launey & Hedgecock 2001, Genetics 159:255) explains distorted segregation ratios, seen widely in bivalves, and supports the dominance theory of heterosis and inbreeding depression, for which there is substantial evidence. However, the fine scale mapping location and temporal expression of these deleterious alleles has not been well characterized. Previous experiments to map genetic load in the Pacific oyster had relatively poor genomic coverage and analyzed only two time points. We are attempting more complete genome coverage, based on a consensus microsatellite linkage map, evaluating over 90 markers and sampling larvae every day for thirty days post fertilization. Using inbred lines from wild populations and classical crossbreeding experiments, we are analyzing segregation ratios for 50 informative microsatellite DNA markers in larvae at 30 days post fertilization from an F₂ and F₃ hybrid family. We look for deviations from expected Mendelian inheritance ratios, owing presumably to selection against identical-by-descent homozygotes linked to nearby markers, and apply a linked selection model to estimate the number of lethal genes in the wild founders. We see, for example, one distortion on linkage group 9, where microsatellite *ucdCgi189* has only three homozygous genotypes of an expected 18 (N=66). We are then following the distorted markers back in time to determine when the deleterious alleles to which they are linked were first expressed in development.

MEASURING SUCCESS IN OYSTER REEF RESTORATION: APPLICATION OF STANDARDIZED MEASURES AND TESTS OF APPROACHES

Martin H. Posey (Department of Biology and Marine Biology; UNC-Wilmington), Troy D. Alphin (Center for Marine Science; UNC-Wilmington), Loren Coen (Marine Resources Research Institute; SCDNR), Keith Walters (Department of Marine Science; Coastal Carolina University), Dara Wilber (Grice Marine Program; College of Charleston)

Oysters along the Atlantic and Gulf coasts of North America have supported a historically important commercially fishery. Oysters are also increasingly recognized as key components of coastal ecosystems, providing habitat for transient and resident fauna, affecting particulate concentrations in overlying waters, reducing wave energy along sensitive shorelines and influencing local biogeochemical cycling. With a decline in oysters along much of the Atlantic coast, there is increased attention to restoring reefs through shell planting efforts. However, the lack of long-term monitoring using standardized methodologies that target both population and ecosystem functions has been an impediment to assessing the success of many restoration projects. In 2004, researchers at a SeaGrant-sponsored workshop proposed a set of sampling criteria and methodologies to provide standardized population and ecosystem measures for assessing the success of oyster restoration projects. We are applying these standardized measures to a series of restored reefs of varying ages along the central and southeast North Carolina coast. This includes comparison of selected habitat and population functions to reference reefs as well as examining the influence of seeding (placing hatchery oysters on the reef at the time of creation), varying tidal position (intertidal to shallow subtidal), and landscape attributes, such as shell depth and proximity of adjacent habitat types, in reef

establishment. This project will provide a comparison among different restoration designs as well as providing information on habitat function in a standardized form that can be compared among studies.

UNDERSTANDING CRASSOSTREA GIGAS POPULATION VARIABILITY USING A GENETICS-BASED MODEL

Eric N. Powell (Haskin Shellfish Research Laboratory, Rutgers University), John M. Klinck (Old Dominion University), Dennis Hedgecock (University of Southern California), Eileen E. Hofmann (Old Dominion University)

Survival and recruitment of marine bivalve larvae represent the interplay of environmental, biological, and physiological processes. However, the genetic characteristics of individual members of a population provide an overlay that determines the overall population response to exogenous and endogenous forces. This study describes the first genetically-based model developed for a marine bivalve species, *Crassostrea gigas*, that can be coupled to more traditional models describing animal growth and development. Explicit inclusion of genetic structure allows a mechanistic understanding of variability in growth, survival, and recruitment. The individual-based genetics model simulates generational changes in population genotype as a function of the number of parents and their genotypes. Each individual has 10 pairs of chromosomes, with multiple genes per chromosome and up to 4 alleles per gene. Some genes affect fitness by varying the chance of mortality; among these are homozygous recessives that are always lethal. The model structure allows genetic mutation and recombination, tracks male and female individuals by means of a sex gene, and permits the effective number of broodstock to be a small percentage of the total adult population. Multi-generational simulations show the disappearance of alleles, a decrease in heterozygosity, and a low effective population number, when only a few parents produce successful progeny and maintain the population yearly. Predicted sex ratios match observations, including the shift from male to female dominance with increasing size. Homozygous recessive genes develop in the population through mutation and slowly accumulate until common enough to affect population dynamics, which then restrains further increases.

COPPER IS AN ESSENTIAL NUTRIENT AND POTENTIAL TOXICANT IN THE DIET OF THE SEA URCHIN, *LYTECHINUS VARIEGATUS*

Mickie L. Powell (University of Alabama at Birmingham), W.T. Jones (University of Alabama at Birmingham), V.K. Gibbs (University of Alabama at Birmingham), H.S. Hammer (University of Alabama at Birmingham), J.M. Lawrence (University of South Florida), A.L. Lawrence (Texas A&M University System)

Due to worldwide overfishing, scientists are currently developing stock enhancement and aquaculture techniques for sea urchins. Successful culture of sea urchins requires a formulated feed that fulfills all nutritional requirements. Copper is an essential micronutrient in the diets of most animals. It is a component of many enzymes involved in energy production and in the protection of cells from free radicals. Excessive levels in the diet can be toxic. Small (ca. 13 g wet weight) *L. variegatus* were fed formulated feeds with low, medium, or high levels of copper for 12 weeks (calculated at 6, 47 and 160 ppm; levels based on established dietary levels for other marine invertebrates, supplemented as CuSO_4). Under these experimental conditions, wet weights of individuals fed a medium level of copper were slightly higher (43.2 ± 1.2 ; $P=0.069$) than those fed low and high levels (39.9 ± 1.2 , and 40.3 ± 1.7 g wet weight, respectively). Gonad and gut wet weights were significantly lower ($P<0.003$) in the high copper diet (6.9 ± 2.5 , 7.0 ± 3.6 and 5.0 ± 1.6 g gonad wet weight and 0.97 ± 0.04 , 1.1 ± 0.06 and 0.83 ± 0.04 g gut wet weight for low, medium and high levels, respectively). However, in the absence of copper supplementation survivorship decreased to 87.5% on the low copper feed. These data suggest that dietary copper is essential for normal physiological function but can be toxic at high levels. Supported in part by Mississippi-Alabama Sea Grant Consortium.

MICROSATELLITE ASSESSMENT OF GENE FLOW IN *AMBLEMA PLICATA* IN THE OUACHITA HIGHLANDS OF SOUTHEASTERN OKLAHOMA

Kathleen Reagan (University of Oklahoma, Oklahoma Biological Survey), Caryn Vaughn (University of Oklahoma, Director-Oklahoma Biological Survey)

Appreciation of genetic diversity in unionids is essential to their preservation. Understanding how habitat perturbations, particularly those that fragment populations, affect this genetic diversity will provide information for better management of their natural habitats. We are examining genetic diversity and relatedness within a common mussel species, *Amblema plicata*, from three rivers in the Ouachita Highlands of southeastern Oklahoma that are fragmented to varying degrees, due to impoundments. We sampled *A. plicata* tissue from ten sites across the Little (N=8), Glover (N=1) and MT. Fork (N=1) Rivers. At each of these sites, mussels were sampled from 15, randomly placed, 0.25m² quadrats. Tissue samples consisting of 20-40 µg of mantle tissue were clipped from a minimum of 30 individuals per site. A subsample of mussel individuals was taken from each site for shell thin-sectioning to determine age. Microsatellite markers will be used to examine both genetic diversity and relatedness of *A. plicata* within and between sites. This will enable us to determine the impacts of impoundments on gene flow.

MOLECULAR MARKERS FOR POPULATION STUDIES, SELECTIVE BREEDING AND PARENTAGE ASSIGNMENTS IN THE HARD CLAM *MERCENARIA MERCENARIA*

Kimberly S. Reece* (VIMS, The College of William and Mary), Gail P. Scott (VIMS, The College of William and Mary), Ryan B. Carnegie (VIMS, The College of William and Mary), Lisa Ragone Calvo (Haskin Shellfish Research Laboratory, Rutgers University), Eugene M. Bureson (VIMS, The College of William and Mary), Mark D. Camara (USDA - ARS, OSU - Hatfield Marine Science Center)

The hard clam (*Mercenaria mercenaria*) industry has been heavily impacted by Quahog Parasite Unknown (QPX), which had caused substantial mortality in cultured clams at some sites along the eastern seaboard of the USA. Selective breeding of clams for traits such as disease resistance, enhanced growth rate and low salinity tolerance might provide stocks that significantly improve harvest yields in particular regions. Stocks derived from geographically distinct wild broodstock demonstrate differential susceptibility to QPX, suggesting a genetic basis for disease resistance. Side-by-side field experiments have shown significantly higher QPX prevalence in stocks from Florida and South Carolina compared to that observed in northern stocks (VA, NJ, MA). Unfortunately, genetic improvement efforts in bivalves are hampered by difficulties inherent in maintaining individually reared families in order to address genetic questions, and in developing elite genetic stocks without severe inbreeding depression. Molecular genetic markers can be used to assign individuals to families following field grow-out challenges and to facilitate maintenance of genetic diversity in stocks. For disease selection challenges, families can be mixed in the field and multi-locus genotypes can be used to assign individual progeny to parents or to reconstruct sibling relationships retrospectively. Individuals used for subsequent generation spawns can be selected based on genetic profiles to maximize diversity and genetic profiles of populations and stocks allow examination of genetic differentiation. We have developed both microsatellite and single nucleotide polymorphism (SNP) markers for *M. mercenaria* and are testing them for the ability to do parentage assignments and stock differentiation.

GENETIC CONSIDERATIONS IN BROODSTOCK SELECTION FOR OYSTER REEF RESTORATION

Kimberly S. Reece* (VIMS, The College of William and Mary), Jens Carlsson (VIMS, The College of William and Mary), Jan F. Cordes (VIMS, The College of William and Mary), Ryan B. Carnegie (VIMS, The College of William and Mary), Mark D. Camara (USDA - ARS, OSU - Hatfield Marine Science Center)

Diseases caused by the parasites *Perkinsus marinus* and *Haplosporidium nelsoni* contributed to decline of native *Crassostrea virginica* populations in the eastern USA, and slowed their recovery. Recently, oyster restoration strategies have focused on improving tolerance to these parasites by planting selectively bred strains in Chesapeake Bay. Enhanced parasite tolerance in domesticated stocks might translate to increased chances of survival and reproductive success relative to wild populations. In addition, disease resistance genes from selected strains might introgress into wild populations through interbreeding. Genetic variation, however, is typically greatly reduced in hatchery lines compared to natural populations and depending on genotypic profiles, as well as relative census and effective population sizes, interbreeding with planted hatchery strains could reduce diversity in wild populations. Oyster populations in the Bay might become genetically less resilient in the face of future disease or environmental challenges. Understanding the genetic mechanisms of disease resistance and interactions between deployed hatchery stocks and wild populations is key to assessing the impact, positive or negative, of such oyster restoration

strategies. Many hatchery stocks being used for restoration in Chesapeake Bay have unique genetic profiles distinguishing them from local wild populations. We are using molecular markers to evaluate the breeding success of deployed oysters and conducting surveys to examine the impact of disease on various populations and selected stocks. Genotypic data enables us to assign individuals to either local native populations, deployed oyster strains or putative hybrid groups, and disease data provides information on relative disease tolerances of the different groups.

REPRODUCTIVE CYCLE OF THE RAZOR CLAM *SOLEN MARGINATUS* (PULTENEY 1799) IN SPAIN. A COMPARATIVE STUDY IN THREE DIFFERENT PLACES

A. Remacha-trivino (University of Rhode Island)

The reproductive cycle of the razor clam *Solen marginatus* (Pulteney 1799) is studied in three different places of Spain through time. Sampling was performed in the natural beds of: Eo Estuary (NE of Spain) in 1994-95, Santander Bay (NW of Spain) in 1998-99 and Terrón Estuary (SE of Spain) in 1999. In Eo, Santander and Terrón, respectively: phase 0 (resting stage) occurred in July-August, September-October and August-September, phase I (early gametogenesis) happened in: August-October, October-December and September-November, phase II (late gametogenesis) was developed in November-April, January-May and December-April and phase III (reproduction) took place in: May-June, June-August and May-July. Two new approaches oriented to the fast monitoring of the gametogenic cycle of the species based on sequential mean drained soft parts weights obtained by simulation and on the macroscopic observation of the gonad are presented

QUANTIFICATION OF PERKINSUS MARINUS IN THE EASTERN OYSTER *CRASSOSTREA VIRGINICA* USING MODERN STEREOLOGICAL TECHNIQUES

Antonio Remacha-triviño (University of Rhode Island), Dodi Borsay-horowitz (US EPA, Atlantic Ecology Division), Christopher Dungan (Maryland DNR, Cooperative Oxford Laboratory), Ximo Gual-arnau (Universitat Jaume I, Castelló, Spain), Javier Gómez-león (University of Rhode Island), Marta Gómez-chiari (University of Rhode Island)

Dermo disease, caused by the protozoan parasites *Perkinsus* spp., is responsible for high mortalities of bivalves mollusks world-wide. In order to improve the knowledge of the pathogenesis of dermo, accurate techniques to estimate the number of parasites in tissue sections are required. This study is aimed to quantify the number and tissue distribution of different stages of *P. marinus* in a natural population of *Crassostrea virginica* from Wickford (Rhode Island, US) by the application of modern stereology and immunohistochemistry. Mean total number of trophozoites in 8 oysters collected in July 2005, of mean shell length (mean \pm SD) of 101.2 ± 5.2 mm, were (mean \pm SE) 11.80 ± 3.91 million and 11.55 ± 3.88 million for the optical disector and optical fractionator methods respectively. The mean empirical error between both stereological approaches was $3.8 \pm 1.0\%$. Trophozoites were detected intracellularly in the following tissues: intestine (30.1%), Leydig tissue (21.3%), hemocytes (14.9%), digestive gland (11.4%), gills (6.1%), connective tissues (except Leydig and mantle) (5.7%), gonads (4.1%), palps (2.2%), muscle (1.9%), mantle connective (0.8%), pericardium (0.7%), mantle epithelium (0.1%), and heart (0.1%). The remaining 0.6% of trophozoites were found extracellularly throughout different tissues. Percentages of trophozoite stages were (mean \pm SE): large, log-phase trophonts (*i. e.* signet-rings): $97.0 \pm 1.2\%$; meronts: $2.0 \pm 0.9\%$; clusters of small, log-phase trophonts (*i. e.* merozoites): $1.0 \pm 0.5\%$. These techniques could be useful to follow parasite distribution and progression in experimental infections, and further explore mechanisms of dermo pathogenesis.

OYSTER HERPESVIRUS 1 (OSHV-1) INDUCED GENES IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*

T. Renault (IFREMER), Faury N. (IFREMER), Barbosa-solomieu V. (IFREMER), Moreau K. (IFREMER), Brunetière C. (IFREMER), Saulnier D. (IFREMER), Pepin J.-f. (IFREMER)

Since 1972, several herpes-like virus infections have been reported among different bivalve species around the world. Viral detection was often associated with high mortality rates in spat and larvae of bivalves, including Pacific oyster (*Crassostrea gigas*). The genome characterization of the virus infecting *C. gigas* allowed it to be included in the Herpesviridae family as an unassigned member and named Osyster Herpesvirus 1 (OsHV-1). The specific objective of the present study was to search for OsHV-1-induced genes expressed in adult *C. gigas* oysters. The suppression subtractive hybridisation (SSH) technique has been used. Oyster haemocytes in contact with infected and non-infected ground larvae were used to extract RNA. Briefly, cDNA was obtained from 1 µg of each hemocyte RNA (control and after virus contact) using the SMART PCR cDNA Synthesis Kit (Clontech), which allowed the full-length amplification of cDNA from mRNA transcripts. A SSH assay was then performed using the PCR-Select cDNA Subtraction Kit (Clontech). The forward-subtracted sample (genes present or up-regulated in presence of the virus) was then used in PCR to amplify the differentially expressed sequences. Cloned PCR products were sequenced using a 3100 Avant Genetic analyser ABI prism (Applied Biosystem). BlastX analysis of the sequenced differentially regulated clones resulted in unique homologues being identified of which some have never been reported previously in any bivalve species. One of the promising candidates corresponds to a homolog with glypican 6 precursor genes. Glypican 6 is a cell surface proteoglycan bearing heparan sulfate. Heparan sulfate proteoglycans serve as cellular receptors for herpesviruses.

HERPESVIRUSES INFECTING BIVALVES : A REVIEW

T. Renault (IFREMER)

Particles morphologically similar to herpesviruses were first detected in an invertebrate (the Eastern oyster, *Crassostrea virginica*) in 1972. Herpes-like viruses have since been identified in various marine bivalve species throughout the world, including the Pacific oyster *Crassostrea gigas*, European flat oyster *Ostrea edulis*, Antipodean flat oyster *Ostrea angasi*, Chilean oyster *Tiostrea chilensis*, carpet shell clam *Ruditapes decussatus*, Manila clam *Ruditapes philippinarum* and great scallop *Pecten maximus*. Infections are often associated with sporadic episodes of high mortality among larvae and juveniles. PCR-based diagnostic methods have facilitated epidemiological investigations showing that healthy adult animals can harbour the viral genome. Transmission experiments have demonstrated the pathogenicity of the virus, and indicated that a single species is probably responsible for all the infections observed. The virus isolated from infected *C. gigas* larvae has been formally classified as a member of the Herpesviridae under the name ostreid herpesvirus 1 (OsHV-1). Characterization of the genome yielded an estimated size of 207 kbp. The capsid morphology and genome sequence of OsHV-1 have been studied in order to assess its phylogenetic status in relation to vertebrate herpesviruses. The conserved gene that comes closest to being herpesvirus-specific encodes the putative ATPase subunit of the terminase, an enzyme complex involved in packaging viral DNA into preformed capsids. Moreover, similarities between the two groups in capsid structure and mechanisms of capsid maturation tip the balance of evidence in favour of a common origin.

ASSESSMENT OF THREE STAINS INTERNALLY MARKING MOLLUSK SHELLS OF CONCHOLEPAS CONCHOLEPAS AND MESODESMA DONACIUM

José M. Riascos V (Universidad de Antofagasta, Investigaciones Oceanológicas), Nury Guzmán (Institut de Recherche pour le Développement, Bondy Cedex, FR), Jürgen Laudien (Alfred-Wegener Institute for Polar and Marine Research), Olaf Heilmayer (Alfred-Wegener Institute for Polar and Marine Research), Marcelo Oliva (Universidad de Antofagasta, Investigaciones Oceanológicas)

Different stains have been used to internally mark the calcified structures of mollusk shells and thereafter for the validation of growth estimates. As an interspecific variation in marking success is known, an assessment of suitability is necessary. The potential of Calcein, Alizarin red and Strontium-chloride-hexahydrate (SCH) was investigated for the gastropod *Concholepas concholepas* and the surf clam *Mesodesma donacium*, two mollusks of commercial importance in Chile. Wild specimens collected off Antofagasta, Northern Chile, were marked using two concentrations of each stain and two immersion periods as well as a control. After immersion, animals were reared for 20 days to allow growth. Mortality, body condition and growth were measured to assess the effects of treatments. For the detection of the incorporated marks, individuals were culled and shell sections analyzed under a fluorescence microscope (Calcein, Alizarin) and a scanning electron microscope (SCH), respectively. The treatments did not show significant effects on mortality and body condition. However, the growth rate of *C. concholepas* was

significantly different ($P < 0.05$) among stains (SCH<Alizarin<Calcein). Calcein markings were successful in all treatments (50 and 100 mg l-1, 3 and 6 hrs.) whereas Alizarin only yielded comparable results with 100 mg l-1 and 6 hrs. SCH just produced faint marks, even at high concentrations. Markings in *C. concholepas* were clearer compared to *M. donacium*, which can be attributed to the gastropod's fast growth rate (0.43 $\mu\text{m d}^{-1}$). Calcein was assumed to be the best marker and did not considerably affect the viability of the species studied.

INTERACTIONS BETWEEN THE SURF CLAM *MESODESMA DONACIUM* AND THE COMMENSAL POLYCHAETA *POLYDORA BIOCCIPITALIS* IN NORTHERN CHILE

José M. Riascos V. (Universidad de Antofagasta, Investigaciones Oceanológicas), Olaf Heilmayer (Alfred-Wegener Institute for Polar and Marine Research), Marcelo Oliva (Universidad de Antofagasta, Investigaciones Oceanológicas), Jürgen Laudien (Alfred-Wegener Institute for Polar and Marine Research)

Although the surf clam *Mesodesma donacium* is one of the most important species for shellfisheries in Chile and the infestation by the symbiotic polychaete *Polydora bioccipitalis* reaches 31.4 %, nothing is known about this species association. Monthly samples from Hornitos (Northern Chile) were collected to determine the shell length (anterior-posterior) when commensalism first occurs, and its effect on the body condition index (BCI = dry tissue mass/internal cavity volume*100). The temporal pattern of the symbiosis was studied by measuring the thickness of the blisters walls formed in the bivalve shell as an estimation of duration of the association. In addition, a laboratory experiment was performed to assess if the commensal relationship affects the growth rate of the host. Preliminary results indicate that the symbiosis can only be found in clams larger than 33.3 mm. As *M. donacium* undergoes a migration from the intertidal (juveniles) to the lower subtidal (post-juveniles and adults) during its life cycle, it appears that the symbiosis only occurs during a later ontogenetic stage when individuals inhabit the subtidal level. BCI was significantly different ($P < 0.05$) between hosting and non-hosting clams. Although the internal shell cavity was smaller in hosting clams, thus affecting considerably the BCI, the proportion of dry tissue mass was not significantly different. This suggests that *M. donacium* is well adapted to the commensal. Thickness of blister walls showed differences (t-test, $P < 0.05$) between months: thinner blister walls were found in May, June and July, suggesting a seasonal pattern of the commensalism.

GENETIC STRUCTURE OF OYSTER REEFS ALONG THE ALABAMA GULF COAST

Gillian Richard (University of South Alabama), Dr. Charles Brockhouse (University of South Alabama)

The oyster bed productivity in areas such as Mobile Bay has abruptly declined in recent years despite the absence of fishing pressures. These declines have negatively affected the commercial and recreational fisheries in Alabama and may have also influenced the Bay's natural ecosystem in unknown ways. In an attempt to increase oyster stocks in Mobile Bay, a restoration program has been initiated. To effectively manage an oyster reef restoration project an understanding of the genetic structure of the oyster population(s) and hence, the extent of local larval transport and genetic exchange among the oyster reefs in the natural environment is of considerable practical importance. This study has established a genetic map of the region by investigating the genetic variation and differentiation among five oyster reefs spanning the Alabama gulf coast. The results indicated high levels of genetic diversity within sampled reefs and F_{ST} revealed little genetic differentiation among the samples. An estimator of gene flow indicated that the main commercial oyster reef, Cedar Point Reef, is the primary source of recruits for the area.

CFD ANALYSIS OF SHELLFISH AQUACULTURE GEAR USED IN INTER-TIDAL AND SUB-TIDAL LOCATIONS

John Richardson (Blue Hill Hydraulics Incorporated), Carter Newell (Great Eastern Mussel Farms), Dror Angel (Massachusetts Institute of Technology), Tessa Getchis (Connecticut Sea Grant Extension Program), Andy Suhrbier (Pacific Shellfish Institute), Jonathan Davis (Tayloer Resources Baywater, Inc.), Daniel Cheney (Pacific Shellfish Institute)

An environmental and technical assessment of alternative methods used to cultivate bivalve shellfish was completed as part of the National Marine Aquaculture Initiative (NMAI). Included in the list of study sites were locations in



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Washington State and Connecticut where aquaculture is being carried out in inter-tidal and sub-tidal waters using bag-on-bottom, bag-on-rack, net-protected or cage culture methods.

To evaluate the effectiveness of these methods used for shellfish aquaculture and to better understand their effect on the local environment, experimental and numerical studies of culture unit hydrodynamics were completed. These studies involved the acquisition of near-field velocity measurements, dye studies, and construction of three-dimensional numerical models based on computational fluid dynamics (CFD) techniques.

Of particular interest are the use of fractional volume techniques to model nets and bags, and the use of a kinetics algorithm to calculate phytoplankton consumption within the culture units. These techniques allow the practitioner to accurately simulate three-dimensional flows through the different types of culture units and to estimate food availability within the culture units.

The results of the modeling were used to help provide explanation of the field data sets, to identify “holes” in the data sets, and to compare the performance of the different shellfish aquaculture practices. The methods developed for this study can also be used as an aid to evaluate siting concerns and to customize gear designed to work optimally at specific locations.

LINKAGES BETWEEN CELLULAR BIOMARKER RESPONSES AND REPRODUCTIVE SUCCESS

Amy H. Ringwood (University of North Carolina - Charlotte), Charles J. Keppler (Marine Resources Research Institute, SCDNR, Charleston, SC), Jennifer Hoguet (College of Charleston, SC), Michel Gielazyn (University of South Carolina, Columbia, SC)

An important issue for cellular biomarker assessments is whether they are related to higher level responses, e.g. are there linkages between cellular and organismal responses that could translate into population level effects? The purpose of these studies was to evaluate if there were relationships between lysosomal destabilization or glutathione concentrations and gamete viability of oysters, *Crassostrea virginica*. Oysters were collected from reference and polluted field sites during the peak spawning period (May-June). Hepatopancreas (e.g. digestive gland) tissues were dissected and a portion was used immediately for lysosomal destabilization assays (based on neutral red retention), and glutathione concentrations were also determined for both hepatopancreas and gonadal tissues. Eggs and sperm from the same adults were also used to conduct embryo development assays with reference seawater collected from a clean site, site water, and also a range of Cd concentrations (the Cd exposures were used to determine if there were differences in susceptibility to pollutants). Baseline embryo development success (e.g. percent normal development when the assays were conducted with reference seawater or site water) was related to lysosomal destabilization, but not glutathione status. However, the susceptibility of embryos to metal exposures was related to glutathione status, i.e. sensitivity to Cd exposures increased with decreasing glutathione levels. These studies support the hypotheses that there are linkages between these cellular biomarker responses and embryo development success and susceptibility to pollutant stress. These kinds of effects on reproductive success could lead to subtle but significant long-term effects on recruitment and viability of oyster populations.

OXYTETRACYCLINE TREATMENT OF WITHERING SYNDROME IN RED ABALONE (*HALIOTIS RUFESCENS*)

Thea T. Robbins (California Department of Fish and Game), Eric Rosenblum (Department of Environmental Toxicology, U.C. Davis), Ronald Tjeerdema (Department of Environmental Toxicology, U.C. Davis), James D. Moore (California Department of Fish and Game), Carolyn S. Friedman (University of Washington)

Since the mid 1980's the disease withering syndrome (WS) has severely impacted wild and cultured California abalone (*Haliotis* spp.). The causative agent of WS “*Candidatus Xenohaliotis californiensis*” infects

gastrointestinal epithelia. The broad-spectrum antibiotic oxytetracycline (OTC) has been shown to control infections in cultured and broodstock abalone as a therapeutic in medicated feed. We examined the efficacy and pharmacokinetics of OTC in infected red abalone, *Haliotis rufescens*, medicated with an artificial diet containing 1.85% active OTC at a rate of 103.4 mg/kg abalone for 10, 20 or 30 days at 13.4°C. The medication was 97% effective in clearing RLP infections in the 10 day treatment and 100% in the 20 and 30 day treatments. Significant differences were observed in prevalence ($p < 0.0001$) and intensity of infection in post-esophagus ($p < 0.0001$) and digestive gland ($p < 0.05$) of treated abalone compared to untreated abalone. Survival was not appreciably different among treatments due to a thermal refuge from disease expression at low temperature; however during a sequential study at an elevated temperature of 17.3°C, unmedicated abalone suffered higher losses and developed clinical WS at a higher rate than medicated animals. Depletion dynamics in abalone tissues showed animals medicated for 10 days accumulated significantly less OTC than those medicated for 20 or 30 days with significantly lower OTC levels present in foot muscle samples relative to digestive gland samples ($P < 0.001$). In contrast, at 17.3°C abalone only accumulated half as much OTC as those at 13.4°C suggesting that both temperature and duration of medication are important regulators of OTC pharmacokinetics in abalone.

OVERVIEW AND APPLICATION OF BAY SCALLOP GENOMIC RESOURCES

Steven Roberts (Marine Biological Laboratory)

The bay scallop (*Argopecten irradians*) is a commercially important bivalve mollusk that supports culturally significant recreational fisheries along the East Coast of the United States. The bay scallop is also an excellent scientific model to study visual signal transduction, early development, and muscle physiology. Furthermore, like other bivalves, scallops are essential components in maintaining sustainable marine environments. In order to provide basic molecular tools to study scallop biology several approaches have been taken including differential display, degenerative primer based RT-PCR and small-scale expressed sequence tag projects. Several gene products from these combined efforts have been further characterized using comparative analysis of gene expression, *in silico* procedures, and recombinant protein systems. Expressed sequence tag derived genetic markers have been developed and used to evaluate stock enhancement. An overview of the molecular techniques employed will be presented including suggestions on how to develop similar efforts. Experimental results will be presented on transcripts associated with scallop early development and growth. This research was supported by the United States Department of Agriculture (grant #2003-35206-12834) and the County of Barnstable Massachusetts.

CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED GENES FROM QPX: INSIGHT INTO POSSIBLE VIRULENCE MECHANISMS

Steven Roberts (Marine Biological Laboratory), Roxanna Smolowitz (Marine Biological Laboratory), Christina Romano (Marine Biological Laboratory)

Quahog Parasite Unknown (QPX) is a protist, genetically identified as belonging to the family Thraustochytridae in the phylum Labyrinthulomycota (slime mold). It proliferates by endosporulation and has been identified as a significant cause of hard clam mortality in the Northeast United States. QPX causes significant disease and mortality in both cultured and wild clam populations in the region. In order to begin to understand what genes are present in the extracellular proteins of QPX mucus and are associated with QPX virulence; we have started to characterize genes that are expressed in QPX under different conditions. In one experiment focused on temperature influences on gene regulation, QPX cultures were grown at 10°C and 21°C for 10 days. Following incubations, total RNA was extracted from cultures and several differentially expressed genes were identified using a differential display polymerase chain reaction technique (GeneFishing DEG System - Seegene). To our knowledge, these are all novel gene homologs in QPX as only rDNA sequences have been previously characterized. One of the genes upregulated at 10°C is a potassium channel protein, based on DNA sequence homology. Similar proteins have been shown to be important for other pathogens as they can inhibit phagocytosis. Additional transcripts and comparative gene expression analysis in QPX strains under different environmental conditions will be presented. The long term goal

of this research is to provide resource managers with basic information on QPX biology that could be used to facilitate hard clam aquaculture and protect wild populations.

RECRUITMENT AND SUCCESSION VARIABILITY IN OYSTER FOULING COMMUNITIES IN BAHIA SAN QUINTIN, BAJA CALIFORNIA, MEXICO

Laura F. Rodriguez (University of California, Davis)

Bahia San Quintin is a shallow bay on the Pacific coast of the Baja California peninsula, Mexico. Historically dominated by soft sediment systems, the bay currently supports a locally important oyster aquaculture industry. In this system, oysters (*Crassostrea gigas*) are cultivated on ropes and remain suspended in the water column for about one year, until they attain market size. These systems provide a significant amount of available substrate for the development of diverse fouling communities, which are dominated by ascidians, sponges, bryozoans, and hydrozoans. These fouling communities harbor non-indigenous species, one of which is the Australian ascidian *Microcosmus squamiger*. This ascidian can attain pest-like densities, completely covering oyster ropes and likely limiting the amount of food and dissolved oxygen accessible to the oysters. Oyster farmers view fouling communities as a nuisance that both harm their oyster crop and increase their handling time in cleaning and packaging oysters. To investigate the impact of these fouling communities on the oyster crop I have been taking monthly recruitment and community succession samples at three sites within Bahia San Quintin for the past two years (2004-2005). I used oyster shells as substrate panels to look at the abundance and prevalence of fouling organisms after 1, 2, 3, 6, and 12 month soaking periods. Results from this study establish recruitment patterns and variability, and community development and succession trends following recruitment. These results, coupled with seasonal and water temperature information, will help develop management options to mitigate impacts of detrimental fouling species.

OYSTER RESTORATION IN AN URBAN LANDSCAPE: CHARACTERIZATION OF A BASIN-WIDE OYSTER POPULATION IN THE LYNNHAVEN RIVER, VIRGINIA

P.G. Ross (VA Institute of Marine Science, College of William & Mary), M.W. Luckenbach (VA Institute of Marine Science, College of William & Mary), A.J. Birch (VA Institute of Marine Science, College of William & Mary), L.D. Coen (Marine Resources Research Institute, SC Dept. Nat. Resources)

Historically in the Chesapeake Bay, quantitative evaluations of oyster reef restoration have focused on subtidal reefs utilizing fisheries-based metrics (e.g. abundance of market-sized, ~76 mm, oysters). Monitoring is typically initiated after restoration activities have been implemented, with few instances of coordinated data collection before, during and after restoration activities. Beginning in 2006, a large-scale oyster restoration effort, including habitat and brood stock enhancement, is planned for the Lynnhaven River Basin, a small and relatively closed tidal sub-tributary of the lower Chesapeake Bay. In contrast to most other restoration sites in this region, the Lynnhaven Basin is heavily urbanized with extensive portions of the shoreline covered with stabilization structures such as concrete, wood or metal bulkheads and granite or concrete rubble that can be considered “non-traditional” habitats, which support substantial oyster densities. This type of habitat poses significant challenges for determining initial oyster population size within the basin and basin-level effects of subsequent restoration efforts. We report on characterization of the basin-wide oyster population (overall abundance and size distribution) prior to the fore mentioned restoration activities, including data collection techniques (e.g. sub-meter accuracy surveying Global Positioning Systems [GPS], integrated with an ArcView-based Geographic Information System [GIS]), and challenges regarding extrapolating oyster data to such a large region. Additionally, we describe the extent and relative proportion of varied habitats in this system along with habitat-specific oyster population parameters.

ROTATIONAL AREA MANAGEMENT IN THE NORTHWEST ATLANTIC SEA SCALLOP FISHERY: ARE CURRENT MANAGEMENT STRATEGIES FLEXIBLE ENOUGH?



**Abstracts of the 98th Annual Meeting
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David B. Rudders (Virginia Institute of Marine Science), William D. Dupaul (Virginia Institute of Marine Science), Noëlle Yochum (Virginia Institute of Marine Science)

Limiting fishing activities in certain areas has gained support as a method to conserve and enhance marine resources. Amendment #10 to the Sea Scallop Fishery Management Plan formally established area rotation as a regulatory strategy to protect aggregations of pre-recruit scallops to optimize gains in yield-per-recruit. The criterion for establishing and re-opening rotational closed areas is dependent upon estimates of the year-over-year growth expressed as a percentage increase of scallop biomass. This strategy is advantageous when large recruitment events are episodic in nature, resulting in a predominant year class. It is also effective because, historically, sea scallop recruitment has been highly variable. Recently, regular recruitment has been observed in the mid-Atlantic region, including the Elephant Trunk Closed Area (ETCA), which was established in July 2004 to protect a large 2002 year class. To evaluate this trend, a fixed grid survey was conducted in October 2005 using both a commercial and a NMFS survey dredge. Results indicate that while the 2002 year class that prompted the closure is present, additional recruitment events have taken place. The addition of these recruits complicates the use of biomass thresholds as triggers for closing and opening rotational management areas. The ETCA is scheduled for a limited opening in 2007. Managers have expressed concern that yield-per-recruit objectives will not be met and there is potential for discard related fishing mortality. We examined the flexibility of the criterion for rotational area management and various spatial and temporal harvesting strategies to optimize fishery yields and minimize non-harvest mortality.

THE EFFECT OF CONDITIONING HATCHERY-REARED GREEN SEA URCHINS TO LOW SALINITY ON SURVIVAL AND GROWTH IN THE FIELD

Michael P. Russell (Villanova)

Harvest data show a precipitous decline in the green sea urchin fishery (*Strongylocentrotus droebachiensis*) in the Gulf of Maine. This situation has prompted studies aimed at enhancing standing stocks by seeding formerly productive fishing grounds with hatchery-reared juveniles. One complicating factor is that these environments are subject to fluctuating salinity. Echinoderms are osmoconformers and generally do not tolerate brackish water. However, green urchins are exceptional in their abilities to acclimate to periodic exposures to low salinity. We conducted a field study to assess the efficacy of conditioning hatchery-reared juveniles to hyposaline conditions prior to releasing them at sites where low-salinity conditions occur. Sea urchins were raised from larvae in the lab and grown from settlement for 15 weeks. This cohort was divided into two groups of equal number and size distribution, and randomly assigned to control and treatment groups. The treatment was subjected to five bouts of low salinity (21‰) for 36 hours every two weeks. As expected, at the end of the acclimation period, the treatment group was significantly smaller than the control. A 2x2 factorial ANOVA design was used to establish these samples in a field experiment with site and treatment level as factors in a sea urchin lease-site in the Piscataqua River, New Hampshire. Growth and survival were monitored for one year and the results show no difference in survival but a significant effect of conditioning on growth. Future efforts to establish seeding programs using hatchery-reared juveniles would benefit from incorporating salinity-conditioning prior to release.

IMPROVED CULTURE TECHNIQUE FOR EDIBLE SEA URCHINS, STRONGYLOCENTROTUS INTERMEDIUS LARVAE

Yuichi Sakai (Hokkaido Institution of Mariculture), Yasuko Konda (Hokkaido Institution of Mariculture), Saburoh Yanagisawa (Hokkaido Aquaculture Promotion Corporation), Hiroyuki Abe (Hokkaido Aquaculture Promotion Corporation), Tsutomu Nishimura (Hokkaido Aquaculture Promotion Corporation), Mitsutake Omoteya (Hokkaido Aquaculture Promotion Corporation), Makoto Azumi (Hokkaido Aquaculture Promotion Corporation)

The artificial mass production technique for *Strongylocentrotus intermedius* developed in 1985 allowed the production of 6.1 million juveniles in 2002, in the region of Hokkaido, Japan. However, many problems still persist particularly during the larval stage, such as mass mortality (39-100%) as measured the sinking of larvae in early 6 and 8 arms stages and the precarious settlement rate (39-98%). Other problem is the necessity for daily cleaning of the larval rearing tank. To reduce these problems, increase in the aeration volume and food availability

by temporal stop of water flow (8hours) was attempted. To study the settlement rate, a new criterion was developed to judge the timing of larvae settlement. With the improved technique, the survival and settlement rate increased 1.7 times and 1.1 times, respectively. With this, the total amount of larval food (*Chaetoceros gracilis*) reduced and the daily cleaning of the rearing tank could be stopped, thus, reducing the load in hatchery operation. The cost of juvenile production reduced by about 10%.

AN INVESTIGATION OF CILIATE XENOMAS IN CRASSOSTREA VIRGINICA

Emily Scarpa (Haskin Shellfish Research Laboratory, Rutgers University), Susan Ford (Haskin Shellfish Research Laboratory, Rutgers University), Bruce Smith (New Hampshire Fish & Game), David Bushek (Haskin Shellfish Research Laboratory, Rutgers University)

Since the late 1990s, unusually high prevalences of xenomas have been noted during routine histological examination of oysters from Great Bay, New Hampshire. Xenomas are formed when intracellular parasites accumulate within host cells, causing them to hypertrophy. Although in fish xenomas are commonly caused by microsporidians, in oysters xenomas are caused by ciliates, genus *Sphenophrya*, and are rare. Because they are macroscopically visible on gills, the marketability of infected oysters has been questioned. In this study, samples were collected every fall from 1997 through 2005 and processed using normal histological procedures. In 2005, counts were also made of macroscopically visible xenomas. Prevalence varied according to site within Great Bay and also by year. In histological sections, it has increased notably since 1997, when only 1% of oysters were affected. In 2004, prevalence ranged from 33% to 82% between sites. Densities were mostly below 20 xenomas per histological section, but reached as high as 173. Macroscopically, samples from 2005 contained a mean 15.5 xenomas per oyster, ranging from 0 to more than 100. The xenomas were located in gill water tubes and were often large enough to occupy the entire cross sectional area. They cause localized epithelial erosion and most likely impede water flow. Nevertheless, the histological appearance of the remaining tissues was not obviously affected and there was no clear correlation between oyster size and infection. There was an inverse relationship between the prevalence of *Haplosporidium nelsoni* (MSX) infections and that of xenomas, although the reason is presently unclear.

A HISTOLOGICAL INVESTIGATION OF OYSTER PARASITES AND PATHOLOGY IN CHINA

Emily Scarpa (Haskin Shellfish Research Laboratory, Rutgers University), Susan Ford (Haskin Shellfish Research Laboratory, Rutgers University), Ximing Guo (Haskin Shellfish Research Laboratory, Rutgers University), Lisa Ragone Calvo (Haskin Shellfish Research Laboratory, Rutgers University), David Bushek (Haskin Shellfish Research Laboratory, Rutgers University)

The proposed introduction of *Crassostrea ariakensis* into Chesapeake Bay as a means to restore oyster populations presents a number of potential risks, such as pathogens and pathological conditions that require careful examination and research before approval. Parasites occurring, even at low prevalence, in *C. ariakensis* within its native distribution may seriously impact this or other species in a different environment. Pathogens present in oyster species coexisting with *C. ariakensis* in its native habitat may also present problems if they are able to use *C. ariakensis* as a host or reservoir. In this survey, 27 samples were collected from 8 provinces along China's coastline. Cross-sections of individual oysters were preserved in Davidson's fixative. Genetic analysis of ethanol preserved gill samples was conducted to identify species of individual oysters. Nine samples were selected based on presence of *C. ariakensis*. These oysters, representing 6 sites and consisting of *C. ariakensis* and a number of coexisting species, were processed by normal histological procedures and examined. Individual *C. ariakensis* were also examined using a fluorescence immunostaining technique to identify presence of *Perkinsus* sp. infections. Observed parasites included ciliates, such as *Sphenophrya*-like ciliates and trichodinids; crustaceans, including intestinal copepods; coccidians, including *Nematopsis* and a coccidian-like organism; rickettsia/chlamydia-like organisms; and trematodes. Overall prevalence of any particular parasite did not reach above 3% and averaged less than 1%. However, prevalences of coccidian-like and *Nematopsis* species were as high as 45% and 64%, respectively, at specific sites. To date, no evidence of a significant pathological impact has been observed.



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GONAD PRODUCTIVITY, COLOR AND TEXTURE IN *STRONGYLOCENTROTUS FRANCISCANUS* FED THREE PREPARED DIETS

Susan C. Schlosser (University of California Sea Grant Program), A. L. Lawrence (Texas A & M University), S.A. Watts (University of Alabama), P.D. Tom (University of California, Davis), J.M. Lawrence (University of South Florida)

Commercial sized *Strongylocentrotus franciscanus* (mean size 104.2 mm TD, 434.4 g) were collected from fishing grounds. An initial sample was dissected immediately. The remainder were placed individually in 25 l aquaria (n=8/treatment) and fed one of three prepared diets. The fourth group was unfed. Dry body compartment indices, daily dry feed intake, gonad color, hardness, and resilience were measured after 60 days. A group of processed, Grade A gonads were compared with laboratory gonads. Results were compared by One Way ANOVA. There were no significant differences for dry gonad and test index, or L (gonad lightness). Dry gut index was significantly greater in prepared Diets 2 and 3 compared to the unfed treatment. Dry lantern index of the unfed group was significantly lower than the initial sample and all prepared diet treatments. Gonad redness (a) was significantly greater in the initial sample than the unfed group. Gonad yellowness (b) was significantly greater in the commercially processed gonads than in prepared diets 1 and 2 and the unfed group. Hardness of commercially processed gonads was greater than Diet 1. Resilience was significantly greater in commercially processed gonads than diets 1 and 3 and the unfed treatment. Feed intake was significantly different with Diet1 (0.45g/urchin/day) > Diet 2(0.41) > Diet 3(0.31). Sea urchin gonads from Diet 3 were overall most similar to processed gonads in color and texture. Diets had similar protein, fiber, and fat content.

PERKINSUS SPP. AND BONAMIA SPP. INFECTIONS IN *CRASSOSTREA ARIAKENSIS* MAINTAINED IN A FULLY CONTAINED AQUACULTURE SETTING

Eric J. Schott (COMB, UMBI, University of Maryland, Baltimore, MD 21202, USA), José A. F. Robledo (COMB, UMBI, University of Maryland, Baltimore, MD 21202, USA), Mohammad R. Alavi (COMB, UMBI, University of Maryland, Baltimore, MD 21202, USA), Keiko Saito (COMB, UMBI, University of Maryland, Baltimore, MD 21202, USA), Satoshi Tasumi (COMB, UMBI, University of Maryland, Baltimore, MD 21202, USA), Wolf T. Pecher (COMB, UMBI, University of Maryland, Baltimore, MD 21202, USA), Gerardo R. Vasta (COMB, UMBI, University of Maryland, Baltimore, MD 21202, USA)

In the Chesapeake Bay, diseases caused by *Perkinsus marinus* and *Haplosporidium nelsonii* (Dermo and MSX, respectively) have contributed to drastic declines of populations the native oyster, *Crassostrea virginica*. The Asian oyster, *C. ariakensis*, which grows readily to market size in Dermo-endemic Chesapeake Bay waters, is being considered for introduction to restore oyster populations. While apparently tolerant to *P. marinus*, *C. ariakensis* exposed to Bay waters may reach prevalences of up to 80%, raising the possibility that it could serve as a reservoir in which the parasite could increase its virulence. A crucial question to address is whether *P. marinus* can be transmitted from *C. ariakensis* to naïve *C. virginica*. We conducted cohabitation experiments with *Perkinsus*-infected *C. ariakensis* and *Perkinsus*-free *C. virginica*. The prevalence of *Perkinsus* infection in both 'donor' and potential 'recipient' populations was assessed at 2 and 4 weeks by PCR-based methods. After 4 weeks, *Perkinsus* was present in *C. virginica*.

A potential protozoal disease of *C. ariakensis*, *Bonamia ostreae*, has been associated with mortalities of experimental populations of *C. ariakensis* in Pamlico Sound of NC. The potential exists for *Bonamia* sp. to be present in Chesapeake Bay. We previously detected PCR amplicons indicative of *Bonamia* spp. in *C. ariakensis* reared in the Chesapeake Bay. We are conducting cohabitation studies in which *B. ostreae*-infected *Ostreaa edulis* are co-cultured with *C. ariakensis* for 4 weeks. We are currently in the process of analyzing possible transmission using PCR-based methodologies. (Supported by grants from the Maryland DNR and NOAA)

THE CALIFORNIA BAREFOOT ECOLOGIST PROGRAM: A COOPERATIVE STOCK ASSESSMENT MODEL FOR THE CALIFORNIA RED SEA URCHIN FISHERY

Steve Schroeter (Marine Science Institute, Univ Calif, Santa Barbara), John Duffy (California Dept. Fish and Game, Retired), Peter Halmay (Commercial Sea Urchin Diver)

The red sea urchin fishery in California is currently managed by a combination of size limits, seasonal and area closures, and a restricted access program. This resource is largely assessed through fishery dependent measures (i.e. landings summarized in 10 nm x 10 nm blocks and port samples of size distributions of catches) and a small number of projects that have gathered fishery independent data. Recent ecological work indicates that fishery dependent measures are not up to the task of stock assessment and that work in the fishery independent realm does not adequately take into account the meta-population structure or the extreme spatial patchiness of the resource. This shortcoming is due in large part to the logistical requirements dictated by a resource with extreme spatial patchiness at scales ranging from 10's to 1000's of meters. We discuss a possible solution which involves the design and implementation of a cooperative stock assessment model using fishermen trained as "Barefoot ecologists" to collect data. We describe how fishermen collect data both on the harvest grounds during routine fishing operations, and using the same sampling design, at sites outside the harvest grounds, thus providing a comprehensive fishery independent assessment of the red sea urchin stocks. We use field data to illustrate the sampling problems and to present two possible solutions we have developed to address them.

MOLECULAR MARKERS FOR PARENTAGE ASSIGNMENT IN HARD CLAM (*MERCENARIA MERCENARIA*) STOCKS

Gail P. Scott (VIMS, The College of William and Mary), Mark D. Camara (USDA - ARS, OSU - Hatfield Marine Science Station), Kelly R. Johnson (VIMS, The College of William and Mary), Ryan B. Carnegie (VIMS, The College of William and Mary), Standish K. Allen Jr. (VIMS, The College of William and Mary), Kimberly S. Reece (VIMS, The College of William and Mary)

The hard clam, *Mercenaria mercenaria*, is currently the most important aquaculture species on the east coast of the United States, yet development of selected clam brood stock is still very much in its infancy. An efficient breeding program that maximizes selection intensity, while maintaining genetic diversity, could result in substantial benefits to the industry. Selective breeding in molluscs, however, is complicated by their high fecundity and high variance in reproductive success, which rapidly can result in inbreeding. Because larval molluscs are impossible to physically tag or mark individually, one of the only practical methods of controlling inbreeding is to rear families separately; however, this requires enormous amounts of labor in both the hatchery and field. In addition, separately reared families must be replicated extensively to randomize environmental effects. Alternatively, using molecular genetic markers, families can be mixed for rearing, desired traits can be measured, and individuals can be retrospectively assigned to families based on multi-locus genotypes. We have recently developed both microsatellite and single nucleotide polymorphism (SNP) markers in *M. mercenaria*. Male and female individuals from hatchery strains originating in different regions were used as parents in controlled laboratory spawns to produce 50 distinct full-sib families. Parental tissue and larvae from each of the 50 crosses were preserved to provide progeny samples with independently known parentage for testing the power of the markers for accuracy at pedigree reconstruction.

INTRODUCTION OF THE EUROPEAN SEA URCHIN (*PARACENTROTUS LIVIDUS*) IN A LAND BASED INTEGRATED SYSTEM IN ISRAEL.

Muki Shpigel (National Center for Mariculture, Eilat, Israel), Ingrid Lupatsch (National Center for Mariculture, Eilat, Israel)

The sea urchin, *Paracentrotus lividus*, was introduced as a candidate species in a fish, seaweed and sea urchin integrated system in Eilat, Israel. While fish represent the main product, effluents from fish culture support the growth of the macroalgae *Ulva lactuca* and *Gracilaria conferta*. Both seaweeds were used as a biofilter to remove dissolved nutrients from the water and as food for the sea urchins. Sea urchin performances in parameters such as

growth, survival, food conversion ratio (FCR), protein and energy use, gonad production, and gonad color were evaluated. Growth from spawning to commercial size (45 mm) on the seaweed diet took *ca* 36 months, FCR on a wet weight basis ranged between 5-7, and survival rates from settlement were 70-80%. Three months before harvest, prepared diets are introduced in order to improve raw growth.

MOLTING AND CHANGES IN BODY COMPOSITION OF THE PACIFIC WHITE SHRIMP, *LITOPENAEUS VANNAMEI*, DURING STARVATION

Anthony J. Siccardi III (Texas A&M University System, Port Aransas, Texas), William H. Neill (Texas A&M University, College Station, Texas), Addison L. Lawrence (Texas A&M University System, Port Aransas, Texas), Delbert M. Gatlin III (Texas A&M University, College Station, Texas), Frank L. Castille (Texas A&M University System, Port Aransas, Texas), Joe M. Fox (Texas A&M University, Corpus Christi, Texas)

Starvation studies using the comparative slaughter method contribute to better understanding of animal bioenergetics. To assess compensatory growth, researchers typically starve animals for brief periods. Few studies of shrimp or other crustaceans have assessed changes in body composition upon starvation over periods as long as one month. To determine the effect of long-term starvation, four weight classes (initially weighing 5.51, 7.19, 14.10, and 16.59g per shrimp) of *L. vannamei* were individually held without feeding in 400 tanks (100 shrimp and tanks per size class). Ten shrimp were removed weekly, weighed and then individually analyzed for energy, ash, moisture and protein until mortality was complete. Shrimp continued to molt throughout the study, despite the energy loss and stress involved in the molting process. The daily loss of protein per shrimp was described by the following equation: protein loss (g shrimp⁻¹ day⁻¹) = 0.0045 * W(g)^{0.92}, where W = geometric mean of body weight between the initial and final weights after 28 days of starvation. Daily loss of energy was described as energy loss (cal shrimp⁻¹ day⁻¹) = 37.15 * W(g)^{0.87}. Molting reduced the volume of the starved 5.51 g shrimp by approximately 34%. A reduction in volume also was noted for *L. vannamei* fed below their maintenance protein and energy requirements. The authors believe this loss of volume reflects a tendency toward homeostatic maintenance of tissue water and energy density requisite for functional integrity.

POLYCULTURE OF THE SHRIMP *LITOPENAEUS VANNAMEI* AND THE SEA URCHIN *LYTECHINUS VARIEGATUS*

Anthony J. Siccardi III (Texas A&M University System, Port Aransas, Texas), Addison L. Lawrence (Texas A&M University System, Port Aransas, Texas), Stephen A. Watts (University of Alabama, Birmingham, Alabama)

Polycultures potential has been researched for many species as it represents a potential cost savings to aquaculturists. This study examines the feasibility of *L. vannamei* and *L. variegatus* polyculture. Ninety-six 19-L tanks connected to a semi-closed recirculating seawater system were stocked with one *L. vannamei*. Each tank contained two elevated mesh cages to which 0, 2, or 4 sea urchins/tank were added. Sea urchins were fed *ca* 0.3 grams of a 27% protein research feed per day per urchin. Treatments (N=16) were: 1) 2 urchins per tank plus 0.2 grams per day of a supplemental commercial 45% protein shrimp feed, 2) 2 urchins per tank with no supplemental shrimp feeding, 3) 4 urchins per tank with no supplemental shrimp feeding, 4) 0 urchins per tank with 0.2 grams per day shrimp feed, 5) 0 urchins per tank with 0.4 grams per day shrimp feed and 6) 0 urchins per tank and no supplemental shrimp feeding. Weight gain was similar in shrimp who consumed fecal material from 4 sea urchins to those fed 0.2 grams per day shrimp feed (P>0.05). Weight gain was higher in shrimp who consumed fecal material from 2 urchins plus 0.2 grams per day of supplemental shrimp feed, and was equivalent to shrimp fed 0.4 grams per day shrimp feed (P>0.05). Fecal material alone from 2 urchins was not able to sustain shrimp growth. Data indicates *L. vannamei* is able to utilize sea urchin fecal material which enhances the likelihood of successful commercial polyculture.

LONG-TERMS STUDY OF FEED INTAKE AND GROWTH UNDER CONSTANT TEMPERATURE REGIME IN THE GREEN SEA URCHIN (*STRONGYLOCENTROTUS DROEBAC*)

Sten I. Siikavuopio (Norwegian Institute of Fisheries and Aquaculture Research), Trine Dale (Norwegian Institute of Fisheries and Aquaculture Research), Bjørn-steinar Sæther (Norwegian Institute of Fisheries and Aquaculture Research)

A long-term study of feed intake, somatic growth and gonad growth of green sea urchin, *Strongylocentrotus droebachiensis*, was conducted. Three size groups of sea urchins (initial diameter: 35mm (S), 45mm (M) and 50mm (L)) were held individually in square chambers for 850 days and fed *ad libitum* a formulated moist feed. The animals were kept at constant water temperature (10°C) and under simulated natural photoperiod regime. Feed intake (FI), feed conversion efficiency (FCE), somatic growth, gonad index (%) and gonad colour were measured. The feed intake in all size groups seemed related season, as the feed intake was significantly higher in summer compared to winter. The multiple linear regression show that the somatic growth curves of different size groups had significantly different slopes ($P < 0.05$) ($S = 37.472 + 0.025x$, $M = 44.447 + 0.019x$, $L = 52.286 + 0.01x$, where $x =$ days). S group had the fastest increase in test growth ($P < 0.05$), followed by size group M, and L. There were no significant differences in gonad index, FCR and gonad colour between the groups at the end of the experiment. Overall, our finding suggest that the feeding rates of the adult green sea urchins show a strong correlation with season, possibly due to the annual reproduction cycle.

OSCILLATIONS IN SEA URCHIN POPULATIONS ALONG THE NORWEGIAN COAST

Knut Sivertsen (Finnmark University College, Alta, Norway)

Barren grounds appeared from kelp beds along the Norwegian coast caused by heavy grazing by the sea urchins (*Strongylocentrotus droebachiensis*) during the 1970's and 1980's. In the 1990's kelp re-established in the southern part after the sea urchin populations had declined. No increase of predators or no parasite infection in the sea urchins was registered in the re-established areas.

A hypothesis is proposed that differences in population dynamics of the sea urchins may explain for this pattern. In the southern area mortality and individual growth rates may be higher than in the northern area because of highest temperature in the south. A higher growth rate may cause a shorter life length in the south than in the north. Then a higher and more regular recruitment is needed to stabilize a population in the south. If the population decreases to under critical breeding level, there is a greater chance for the sea urchin population to break down.

A bet-hedging life history strategy is previously proposed for sea urchins. Bet-hedging, explained shortly, means that populations sometimes rapidly increase to a high level, but afterwards the density gradually decreases. This strategy is here discussed in the light of different stages of the life table and regional differences in the water temperature. Each parts of the life cycle should be analysed to find if the mortality at any of the stages is sensitive to fluctuations. High longevity of a species may compensate for high variability in recruitment.

A REVIEW OF QPX DISEASE EMPHASIZING INITIATION AND PROGRESSION

Roxanna Smolowitz (Marine Biological Laboratory)

QPX was reported by Drinnan and Henderson in hard clams from Canada in 1960. In 1995, QPX was diagnosed in cultured hard clams in Provincetown and Duxbury, MA. QPX has since been identified in clams from Virginia, New Jersey, New York and Rhode Island waters, as well as other locations in Massachusetts. It continues to cause marked mortality in MA and RI. QPX organisms primarily invade through the mantle at the base of the siphon. Studies have demonstrated direct infection of naive clams 3 months after exposure to infected dying clams, but not when exposed to cultured QPX via the water column. Infection location in 100% of those clams occurred at the siphon base and in the adjacent mantle. In other work, seed clams examined 6 months after planting showed disease in 33% of the population and the primary infection site, identified histologically, was at the base of the siphon. QPX organisms were noted amid debris in the mantle cavity at the base of the retracted siphon (pseudofeces location). These finding indicate residence time in that location, perhaps as part of pseudofeces, is important in the pathogenesis of the disease. Quizzically, prevalence of QPX disease appears to vary greatly with some plots showing high prevalence in one clam population followed by low prevalence in a replacement population, or some

plots repeatedly showing high prevalence of the disease even in replacement populations. Studies show disease prevalence at any single time period may not accurately indicate previous or future disease prevalence.

GENETIC IDENTIFICATION AND PHYLOGENY OF THE UNIONID GENERA, LAMPSILIS AND ELLIPTIO OF THE SOUTH EAST ATLANTIC SLOPE

Kristine M. Sommer (University of North Carolina Wilmington), Ami E. Wilbur (University of North Carolina Wilmington), Michael A. McCartney (University of North Carolina Wilmington)

Approximately 70% of freshwater mussel species in North America are considered extinct, endangered, or threatened, and a large number of these are endemic to a narrow geographic range. Freshwater mussel conservation efforts have been limited by taxonomic ambiguities and morphologic convergence. Lake Waccamaw in southeastern North Carolina contains two described endemic species, *Lampsilis fullerkati* and *Elliptio waccamawensis*, which conchologically are nearly identical, complicating conservation efforts. To help alleviate this problem, a Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay was developed for genetic identification. Genomic DNA was obtained using a non-lethal method of hemolymph extraction. Two diagnostic RFLPs were used to type 112 individuals. RFLP and sequencing data showed that three individuals were misidentified based on morphology. In addition, phylogenetic analysis was used to assess the taxonomic status of these putative endemics. 16s ribosomal DNA sequences were obtained from 140 individuals from both genera, sampled from Lake Waccamaw, the adjacent Waccamaw River, and the Yadkin/Pee Dee and Lumber Rivers in the Pee Dee Drainage. Results from neighbor-joining and maximum likelihood trees suggest the endemic status of both *L. fullerkati* and *E. waccamawensis* may need to be reconsidered. *Lampsilis fullerkati* individuals fall in a large clade containing *Lampsilis radiata radiata* from outside the lake, and the endemic is not a distinct phylogroup. Additional, faster-evolving gene regions and type specimens are being sequenced to evaluate this result. A similar approach is being used for *E. waccamawensis*, which appears phylogenetically indistinguishable from *Elliptio* collected outside of the Lake.

INFLUENCE OF CLIMATIC CYCLES ON THE INFECTION OF EASTERN OYSTERS BY PERKINSUS MARINUS

Thomas M. Soniat (Department of Biology, Nicholls State Univ.), John M. Klinck (Center for Coastal Physical Oceanography, Old Dominion Univ.), Eric N. Powell (Haskin Shellfish Research Laboratory, Rutgers Univ.), Eileen E. Hofmann (Center for Coastal Physical Oceanography, Old Dominion Univ.)

Perkinsus (= *Dermocystidium*) *marinus* is a major cause of mortalities in eastern oysters, *Crassostrea virginica*. Since initiation of infection and progression of disease are favored by high temperature and high salinity, we hypothesized that climatic cycles influence cycles of disease. Using wavelet analysis and a 10-year data set of disease prevalence and intensity, and water temperature and salinity from a Louisiana site, we show a teleconnection between the El Niño Southern Oscillation and oyster disease in the northern Gulf of Mexico. Inter-annual variation is important in the initiation and intensification of disease, and salinity is the primary driving factor. An increase in salinity is followed in several months by an increase in prevalence followed in several months by an increase in infection intensity, with salinity shifts strongly driven by ENSO events. The time series suggests that epizootics can be initiated within 6 months of a La Niña event, during which high water temperature and high salinity coincide. This relationship makes it possible to anticipate epizootics of *P. marinus* from climate models and to better manage oyster populations.

DERMOWATCH DETECTS DISEASE HOTSPOTS IN THE GULF OF MEXICO

Thomas M. Soniat (Department of Biology, Nicholls State Univ.), Sammy M. Ray (Department of Marine Biology, Texas A&M Univ. Galveston), Enrique Kortright (Kortright Corporation), Lance Robinson (Texas Parks and Wildlife Department)



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DermoWatch (www.dermowatch.org) is a web-based community for the monitoring and management of the lethal oyster parasite, *Perkinsus marinus* (= *Dermocystidium marinum*). Oysters from the major oyster producing areas of the Gulf of Mexico are collected and assayed for *P. marinus*. The web site displays station information, water temperature and salinity, and percent infection and disease intensity (weighted incidence) of sub-market and market-sized oysters.

Weighted incidence values of 2.0 and greater are highlighted as “hotspots”, where oysters (*Crassostrea virginica*) are likely experiencing serious disease-related mortality. Recent disease hotspots are found in Corpus Christi Bay, Aransas Bay, Matagorda Bay and Galveston Bay. Within a bay, hotspots are established on high-salinity reefs and proceed up-estuary. Between bays, hotspots tend to progress from south to north. Within and between bay patterns of progression and regression of disease hotspots are likely cyclical, and should provide a useful indicator of the trajectory of infection.

INFLUENCE OF OYSTER REEF VERTICAL COMPLEXITY IN STRUCTURING SPECIES SPECIFIC INTERACTIONS AND TROPHIC LINKAGES

Joseph Michael Sonnier (UNC-Wilmington), M. H. Posey (UNC-Wilmington), T. D. Alphin (UNC-Wilmington)

Habitat complexity is important in regulating trophic linkages, with broad implications for community composition. Habitat complexity can alter species interactions through impacts on refuge and food. High and low complexity oyster reefs in southeastern North Carolina provide an ideal system to test these ideas. Interactions between bivalve prey (oysters and mussels) and predators (mud crabs) may be affected by the presence of vertical complexity and consequent access by larger predatory crabs. We examined the effects of oyster reef vertical complexity on mud crab populations and mud crab: bivalve interactions in the presence of a top predator, the blue crab. Mud crab density was significantly greater in areas of high vertical complexity during both field and lab studies. Lab studies indicated mud crab predation on bivalve (oyster spat and mussels) prey was greater in high complexity oyster patches, but consumption of mussels was greater than consumption of spat for all trials. In trials containing the top predator, predation by mud crabs was reduced, especially in the low complexity patches where predation approached zero. Field studies show oyster spat recruitment to be higher on newly created low complexity reefs. Tethering trials indicate predation on mud crabs increased as oyster reef complexity decreased with open sand having the highest predation. However, an edge effect was detected for high complexity reefs, with highest predation on mud crabs located within 5 cm of the edge. Reef complexity in this system may have limited top predator access, affecting mud crab: bivalve predator-prey dynamics.

THE FEASIBILITY OF USING TRIPLOID CRASSOSTREA VIRGINICA FOR ON BOTTOM CULTURE IN THE CHESAPEAKE BAY

Melissa Southworth (Virginia Institute of Marine Science), Roger Mann (Virginia Institute of Marine Science), A. Thomas Leggett Jr (Chesapeake Bay Foundation), Aj Erskine (Cownt Seafood Company and Bevan's Oyster Company)

The Federal Register, Vol. 69, No. 2, dated Monday, January 5, 2004 provided notice of “DEPARTMENT OF DEFENSE, Department of the Army; Corps of Engineers Intent [To Prepare a Programmatic Environmental Impact Statement] for the Proposed Introduction of the Oyster Species, *Crassostrea ariakensis*, Into the Tidal Waters of Maryland and Virginia To Establish a Naturalized, Reproducing, and Self-Sustaining Population of This Oyster Species.” and described seven alternatives to be evaluated in the EIS process. Alternative 4 addressed the issue of using the native oyster (*Crassostrea virginica*) species in the development and expansion of aquaculture in the Chesapeake Bay. In the past, the majority of Virginia’s commercial oyster production came from leased oyster ground. Historically, lease holders would either purchase or harvest oyster seed from rivers such as the Great Wicomico, Piankatank or James, and plant on their leases throughout the Virginia portion of the Bay. We describe a project to examine the feasibility of using triploid native spat on shell in the same manner as oyster planters have used wild oyster seed from seed Rivers. Approximately forty million triploid larvae were set on 400 bushels of shell

and planted on an oyster bed owned by Bevan's Oyster Company in the Yeocomico River, Virginia in summer 2005. We present here early results of setting success, growth, mortality and condition index.

COMMUNITY ECOLOGY OF FRESHWATER MUSSEL BEDS: THE ROLE OF TEMPERATURE AND DOMINANT SPECIES ON COMMUNITY STRUCTURE AND RENDERED EC

Daniel E. Spooner (University of Oklahoma), Caryn C. Vaughn (University of Oklahoma)

Recent work suggests that freshwater mussel beds significantly influence stream ecosystem function through benthic-pelagic coupling of energy and nutrients. Mussel communities comprise a mosaic of species that differ in their species-specific thermal performance. These performance curves influence resource acquisition, potentially shaping community structure (species dominance) and subsequent ecological function. We traveled to 21 mussel beds across southeastern Oklahoma and western Arkansas. We quantified community structure (time-search and quadrats), physiological condition (glycogen, body condition index (BCI), and measures of rendered services (metabolism, nutrient excretion). We predicted that species would be dominant at sites that closely resemble their empirically derived thermal performance optima. Our results to date suggest that communities are alternately dominated by two species *Actinonaias ligementina* and *Amblema plicata*. Both species co-occur, have opposing thermal performance trajectories, and are negatively correlated within sites. In addition, condition and performance indices of co-occurring mussel species was related to species richness, *A. ligementina*, and *A. plicata* biomass. These results suggest that species interactions including facilitation/competition may be important factors explaining community structure and subsequent ecological services rendered by freshwater mussel beds.

OVIGERY IN THE SPOT SHRIMP (PANDALUS PLATYCEROS) OF HOOD CANAL

David A. Sterritt (Washington Dept. of Fish and Wildlife)

Egg numbers and seasonal trends in ovigery of were established for Spot shrimp (*Pandalus platyceros*) from Hood Canal during testfisheries conducted during the 2003-2005 season. Ovigerous shrimp were found to be smaller in size and had fewer eggs than Spot shrimp sampled in other investigations. A linear relation was found between egg number and carapace length.

EMBRYONIC DEVELOPMENT AND MORPHOMETRY OF BLUE KING CRAB PARALITHODES PLATYPUS, STUDIED BY IMAGE ANALYSIS

Bradley G. Stevens (NMFS/NOAA Kodiak Fisheries Research Center)

Embryonic development has been described for few commercial crab species, and no standard exists for defining developmental stages. I examined the embryonic development of the blue king crab, *Paralithodes platypus*, from the Pribilof Islands in the eastern Bering Sea. Fertilized embryos were digitally photographed at various intervals throughout their development using a compound microscope and measured with an image analysis program. Seven morphometric parameters were measured (total area, yolk area, embryo length and width, average diameter, eye length and width) and four indices were calculated (percent yolk, ellipticity, elongation, and circularity). First divisions occurred on day 4, after which divisions occurred daily until the blastopore appeared at day 28. A "V□"-shaped embryo became apparent on day 114, and the eyes became pigmented by day 192. Hatching occurred from day 381 to day 409, and required at least 33 days. Embryo area declined from 0.95 mm² on day 1 to 0.83 mm² on day 72, then increased to 1.28 mm² on day 388. Developmental stages were defined visually and by using cluster analysis of embryo measurements. Both methods resulted in an optimum selection of 12 stages. Visual methods were better at defining early changes, but morphometric measurements were better at defining middle and later stages. Morphometric analysis techniques may lead to improved understanding of crustacean embryogenesis and effects of environmental change, and have applications in the aquaculture industry.



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PRELIMINARY ANALYSES OF GENETIC STRUCTURE WITHIN AND AMONG REMNANT POPULATIONS OF THE OLYMPIA OYSTER, *OSTREA CONCHAPHILA*.

David A. Stick (Oregon State University, COMES, HMSC, Newport, OR.), Heather Hunsperger (Eckerd College, St. Petersburg, FL.), Chris Langdon (Oregon State University, COMES, HMSC, Newport, OR.), Michael A. Banks (Oregon State University, COMES, HMSC, Newport, OR.), Mark D. Camara (USDA-ARS, HMSC, Newport, OR.)

The Olympia oyster, *Ostrea conchaphila*, is the only oyster species native to the Pacific Northwest. Historically, the species ranged from Southeastern Alaska to Baja, California and supported both Tribal subsistence fisheries and large commercial harvests. Over-exploitation, habitat degradation, and competition and predation from non-native species have drastically depleted densities and reduced the overall range, but remnant populations persist. Due to the species' historical significance and the ecological services provided by oyster reef habitats, numerous restoration efforts are proceeding without a full understanding of existing population structure, which may be complicated due to *O. conchaphila*'s larviparous mode of reproduction and limited larval dispersal as well as extensive population admixture due to human mediated translocations. Identifying appropriate broodstock and maintaining the genetic integrity of populations is essential for the long-term success of restoration efforts but conducting population-level studies has been hindered by a lack of molecular markers. We have developed a number of microsatellite DNA markers in *O. conchaphila* and conducted preliminary analyses of genetic structure. We first constructed several microsatellite enriched genomic libraries then isolated and sequenced individual clones from them. We next designed primers to the flanking regions of confirmed microsatellites, optimized PCR conditions and verified Mendelian segregation in larvae from 5 hatchery-produced full sib families. Polymorphism was assayed using a panel of 96 oysters from 8 locations, and the most polymorphic markers were used for preliminary analyses of broad-scale patterns of genetic diversity and differentiation within and among populations ranging from northern Vancouver Island, BC to San Francisco Bay, CA.

IMPACTS OF OYSTER REEF ARCHITECTURE ON SPECIES DIVERSITY AND PREDATION

Jennifer L. Stiner (University of Central Florida), Dr. Linda J. Walters (University of Central Florida)

Widely regarded as a keystone species and ecosystem engineer, the eastern oyster *Crassostrea virginica* plays a vital role in estuarine environments. Complex three-dimensional oyster reefs act as biodiversity havens. Recently, concern for this resource has arisen in Mosquito Lagoon, Florida. Since the 1990s, intense boating activity has caused atypical dead margins (mounds of disarticulated shells) to emerge on the seaward edges of oyster reefs located along major boating channels. Once dead margins are formed, little is known about their influence on biotic composition and interactions.

This study focused on the impact of dead margins on (1) species diversity and (2) predation. For one year, monthly surveys were conducted using lift nets to document all mobile species present on reefs in Mosquito Lagoon. Sixty-four species were recorded, including fishes, crustaceans, mollusks, echinoderms, and worms. Mini lift net trials revealed species diversity to be highest on fore-reef and back-reef areas, with drastic reductions on dead margins of impacted reefs.

Field experiments were conducted to determine the impact of dead margins on the vulnerability of juvenile oysters. Structural variables (e.g. shell orientation, single versus clumped shells, and overall slope) were manipulated and effects were observed on oyster mortality and predator maneuverability. Responses of three major predators (*Callinectes sapidus*, *Urosalpinx cinerea*, and *Panopeus herbstii*) differed. For *C. sapidus* and *P. herbstii*, predation was higher on disarticulated shells while *U. cinerea* showed a preference for spat on clumped shells. Overall, this study documents ecological implications of dead margins on reefs of *C. virginica*.

VARIATION IN OYSTER SHELL STRENGTH BASED ON STOCK ORIGIN, SHELL SIZE AND TRANSPLANT EFFECT

Heather Stoker (University of North Carolina- Wilmington), Troy Alphin (Center for Marine Science), Martin Posey (University of North Carolina- Wilmington)

This study compares the breaking strength of *Crassostrea virginica* shells from four Southeastern North Carolina estuarine systems: New River, Cape Fear River, Stump Sound and White Oak River. Shell strength comparisons were made among three size classes within each estuary and among estuaries for oysters in one size class (40-<50 mm). Five oysters per size class per estuary (120 oysters total) were selected. As morphological differences may suggest variation in mechanical properties, the length, width, muscle scar thickness and weight of each shell were recorded. An eleven-millimeter disc was cut from the muscle scar of the right valve of each specimen. The discs were strength tested using an Instron 8511 material testing machine, measuring compressive break strength (MN/m²). The oysters used in this project were from a previous study on stock differentiation; therefore, shell strength can be compared to existing data on the oysters' growth, survival, stock type and estuarine conditions. If the intrinsic strength of *Crassostrea virginica* shells differs among origin or transplant location, this has implications of potential differences in predation risk and susceptibility to disease.

POPULATION STRUCTURE AND PHYLOGEOGRAPHY OF THE PINTO ABALONE (*HALIOTIS KAMTSCHATKANA*) IN WASHINGTON STATE

Kristi Straus (University of Washington), Kerry Naish (University of Washington), Carolyn Friedman (University of Washington)

The Pinto abalone (*Haliotis kamtschatkana*) is the predominant haliotid species in Washington State and is an ecologically important herbivore in rocky subtidal habitats. Although the Washington abalone fishery was closed in 1994, abundance and population densities have declined dramatically since this time. In order to enact an effective conservation strategy, it is imperative to determine the population structure of the Pinto abalone. Previous microsatellite studies indicated low differentiation among Pinto abalone collections in British Columbia, Canada. However, our research on genetic variation at microsatellite loci in over 100 individuals from Washington State revealed substantial population structure. All microsatellite loci exhibited significant deviations from Hardy Weinberg expectations (positive F_{is}) and linkage disequilibrium was evident between most pairs of loci. Using MtDNA sequence analysis, ten individuals from S.E. Alaska (U.S.), British Columbia (Canada), and Washington State (U.S.), and five individuals from California (U.S.) were sequenced at the Cytochrome Oxidase B locus (Cyt B, 404bp). Results from this locus indicate that although animals from Alaska, B.C, and California appear to be closely related, six of the ten Washington individuals sequenced diverge strongly. These six individuals share four haplotypes unique to Washington State. These same six individuals were sequenced at Cytochrome Oxidase I (COI, 343bp). At COI, these six individuals share three unique haplotypes relative to sequence available on Genbank. Further studies are needed to elucidate the biological significance of the observed genetic differences. These findings have significant implications for the listing and restoration of abalone found in Washington State.

A SURVEY OF MARINE COMMUNITIES IN ALTERNATIVE CULTURE METHOD OYSTER AND CLAM SITES.

Andrew D Suhrbier (Pacific Shellfish Institute), Daniel P Cheney (Pacific Shellfish Institute), Jonathan P Davis (Baywater Inc.)

The past two decades have seen a rapid expansion of alternative and innovative methods for the cultivation of oysters and hard-shell clams; however, there is limited information on the interactions of these practices with the surrounding environment. As part of a larger project, multi season sampling was conducted to 1) assess and compare benthic infauna and epifauna species diversity and density, across habitat types within culture and control sites; 2) assess responses of infauna and epifauna to culture conditions; and 3) monitor and compare selected macrofauna including juvenile salmon, shrimp and crabs, across habitat types. Methods evaluated included bag-on-rack, bag on bottom, and suspended bag culture of oysters and bag on bottom and net-protected ground culture of manila clams. Discussed will be the results of seasonal benthic collections, net deployments and underwater video footage at each survey site. Survey sites included commercial shellfish farms in Thorndyke Bay, Hood Canal and Eld Inlet, in South Puget Sound, Washington. This project was funded by the SeaGrant National Marine Aquaculture Initiative.



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CONNECTICUT'S HARD CLAM INDUSTRY AND QPX-DISEASE

Inke Sunila (State of CT, Dept. of Agriculture, Bureau of Agriculture)

Connecticut, on Long Island Sound's north shore, is the leading producer of hard clams, *Mercenaria mercenaria*, with a yearly production of over 400,000 bushels and a yearly farm gate value of over \$10 million. Since 1997, the year of a major *Haplosporidium nelsoni* (MSX) epizootic among oysters, production has been rising steadily. After that harvesters concentrated on the hard clam fishery. Hard clam aquaculture is practiced on over 65,000 acres of leased grounds. Recruitment originates mostly from natural set and harvest size clams are collected by hydraulic dredges and transplanted onto approved grounds for depurification prior to marketing. The health of CT's clams has been monitored annually since 1997. QPX was detected in six out of 2358 clams (0.3%) from 77 different samples along the shoreline. None of the QPX-positive clams originated from commercial clam grounds. Two positive clams were collected from natural oyster beds during culching. Three positive clams were collected next to shipping channels. One of the clams originated from a bed of false quahogs, *Pitar morrhuana*, being surveyed as a potential future hard clam site. Sampling sites for the positive clams are distributed along the entire CT shoreline: Bridgeport, Stratford, New Haven and Groton. Histopathologically affected clams have mucus producing, proliferating presentation of QPX-disease. However, QPX is not currently considered to pose a threat to CT's hard clam industry.

THE LOUISIANA OYSTER RECOVERY PLAN: A RESPONSE TO HURRICANES KATRINA AND RITA

John Supan (Louisiana Sea Grant College Program, LSU), Mike Voisin (Louisiana Oyster Task Force, Motivatiit Seafood, Inc.)

Hurricane Katrina caused unprecedented damage to the central gulf coast, being the greatest natural disaster in U.S. history. The paths of Katrina and Hurricane Rita passed through major Louisiana oyster production areas, causing between 55-70% oyster mortality to the public grounds by scouring and overburden, with likely similar damage to private farms yet to be determined. Infrastructure and fleet damage to public agencies and the private sector was also severe.

The Louisiana Oyster Task Force, legislatively mandated to guide the state in the management and development of its oyster industry, is enacting a recovery plan to address five areas of need: (1) vessel and lock recovery; (2) harvest areas, (3) dock and unloading facilities, (4) processing plants, and (5) market development. This multi-faceted approach challenges all involved to new paradigms. Some of the components include:

- Refloating some 1,800 commercial fishing vessels aground by storm surges;
- Continued and additional microbiological sampling to open more harvest areas;
- Develop dockside refrigeration program;
- Cultch placement on public and private farm lands;
- Open and close oyster seasons on public oyster grounds by location and specific dates;
- Lift current moratorium on new oyster farm lands;
- Rebuild and improve the Sea Grant oyster hatchery on Grand Isle and initiate a Louisiana Oyster Seed Program focusing on genetically superior stocks;
- Develop off-bottom culture in designated marine enterprise zones and/or aquaculture parks; and,
- Postpone 2005 farm rental payments until 2006.

BIOCHEMICAL COMPOSITION AND ADDUCTOR MUSCLE CELL SIZE OF TRIPLOID AND DIPLOID BAY SCALLOP *ARGOPECTEN IRRADIANS*

Amandine Surier (Martha's Vineyard Shellfish Group, Oak Bluffs, MA 02557), Chester B. Zarnoch (AREAC, Brooklyn College, Brooklyn NY 11210), Richard C Karney (Martha's Vineyard Shellfish Group, Oak Bluffs, MA 02557)

Triploidy which has been found to improve overall performance in many shellfish, is particularly interesting in scallops as the increased growth rate is associated with decreased gonad development and increased adductor muscle mass. Triploid's increase in somatic biomass is thought to be the result of energy reallocation from reproduction

towards somatic growth or increased fitness due to higher heterozygosity. Another hypothesis is triploidy gigantism, which associates the increased size of triploid organisms with a larger cell size.

In July 2003, under funding from the Northeastern Regional Aquaculture Center, triploidy was induced in the bay scallop *Argopecten irradians*. The experimental scallops, which tested 97% triploid in July 2004, were grown in bottom cages in Katama Bay, Massachusetts. Triploid wet adductor muscle indexes were 52% and 17% greater than diploids in July and October 2003 respectively.

In December 2004, triploid and diploid adductor muscle samples were sent to Brooklyn College for biochemical composition. Although the protein and carbohydrate contents were higher and lipid content was lower in triploid muscles the differences were not significant. In the spring 2005, survivors from the experiment were sampled for cell size analysis. A portion of the smooth adductor muscle from triploids and diploids was processed using standard histological techniques, photographed and analyzed for muscle cell size and number of cells/area using NIH ImageJ software. Adductor smooth muscle fibers of triploids were significantly wider than those of diploid controls. In contrast the number of muscle fibers per unit area was not significantly different between ploidy groups.

EFFECTS OF PREDATION AND SETTLEMENT SUBSTRATE CHOICE ON THE POST-SETTLEMENT SURVIVAL OF RED ABALONE, *HALIOTIS RUFESCENS*

Clara Svedlund (Ecology, Evolution and Marine Biology, U. C. Santa Barbara), Dr. Steven Gaines (Ecology, Evolution and Marine Biology, U. C. Santa Barbara), Dr. Jennifer Caselle (Ecology, Evolution and Marine Biology, U. C. Santa Barbara), Dr. Laura Rogers-bennett (California Department of Fish and Game & Bodega Marine Lab)

Historically abundant populations of abalone (*Haliotis* spp.) are now at drastically reduced levels throughout Southern California. Two particularly vulnerable stages in the life cycle of these broadcast spawning invertebrates are the larval and post-settlement stages. Laboratory studies of predation on cultured veliger larvae and newly settled red abalone (*Haliotis rufescens*) indicate that predation may be an extremely important source of mortality at these stages. Results from predation experiments revealed that important predators of *H. rufescens* include the crabs *Pagurus* sp., *Cancer* sp., and *Scyra* sp., the terebellid *Eupolyornia* sp., and the sea urchin *Strongylocentrotus purpuratus*. Ninety-seven percent of all predators tested were sources of mortality for settling or settled abalone, whether due to consumption, bulldozing, or other modes. Settlement experiments using three categories of encrusting red algae (ERA) indicated that substrate type may play a larger role in the survival and subsequent growth of settled larvae. Compared to the two other types, settlement and post-settlement survival was significantly higher for the category of crustose coralline algae (CCA) with an irregular surface (e.g. *Lithothamnium pacificum*). Further experiments that tested the interaction of substrate (the three ERA types) and predation (using two different predators) revealed that the more rugose substrate again conferred the highest survival. This higher survival may be due to the morphology of the CCA, which might provide abalone with predation refugia and/or superior grazing sources. Potential larval/post-larval seeding experiments may benefit from selection of sites with a higher cover of the rugose encrusting corallines, and exclusion of probable predators.

ANNUAL VS. NONANNUAL EGG EXTRUSION, MATING VS. UTILIZATION OF STORED SPERM, AND LARVAL HATCHING OF KODIAK, ALASKA DUNGENESS CRAB

Katherine M. Swiney (NOAA Fisheries, Alaska Fisheries Science Center)

Previous studies report nonannual egg extrusion among Dungeness crab in southeastern Alaska which differs from the annual egg extrusion typically reported for Dungeness crab. Dungeness crab from Kodiak, Alaska waters were reared in flow-through tanks to determine if egg extrusion is annual and to observe which crab mate or utilize stored sperm to fertilize egg clutches. Females were collected from 2 bays on the eastside of Kodiak Island May through August 2005, a time in which females should either have eggs or recently hatched larvae. Thirty one percent of females collected did not have eggs the previous reproductive season. Seventy one percent of females brought into the laboratory used stored sperm to fertilize eggs while 26% molted/mated. Females that molted/mated were significantly smaller than females that did not molt. As of 12, December 2005, 52% of females extruded eggs in two consecutive years, whereas 28% skipped at least one reproductive season and extruded an egg clutch the following season; more extrusion is expected. Larval hatching was examined in 2004 with females brought into the laboratory a week before hatching, which began at the end of May and lasted 25 days. Individually, larval hatching

ranged from 8 to 16 days with a mean of 11 days. Larger crab took significantly longer to hatch larvae and had significantly more larvae. Egg extrusion is not annual for all mature Dungeness crab collected from Kodiak waters, however preliminary evidence suggests more crab may extrude eggs annually in the Kodiak area than in southeastern Alaska.

CAROTENOIDS IN THE SEA URCHIN *PARACENTROTUS LIVIDUS*

Rachael Symonds (Liverpool John Moores University, Liverpool UK), Andrew Young (Liverpool John Moores University, Liverpool UK)

The roe of the edible sea urchin *Paracentrotus lividus* is commercially valuable and highly prized in the sushi market. However, roe colour varies with season, the urchin's nutritional state, its state of maturity and its sex. This study has characterised the carotenoid pigment profile of the gonads and gut wall of wild *P.lividus* using a combination of reversed-phase diode-array HPLC and LC-MS.

Gut

The major pigments occurring in the gut wall are breakdown products of fucoxanthin, namely fucoxanthinol and amarouciaxanthin A. Lower levels of echinenone, lutein, isozeaxanthin and β -carotene are also present. The gut wall of the female urchins contains pigments not present in the males, indicating additional metabolism in the former. It is clear that the gut wall is a major site of carotenoid metabolism.

Gonad

Male and female gonads have a broadly similar pigment profile with echinenone accounting for between 70-80% of the total carotenoid (c.f. gut: 20%). Both all-*trans* and 9'-*cis* forms of β -echinenone are found in the ratio 1:10 (w/w). The detection of large levels of 9'-*cis*-echinenone in wild *P.lividus* is unexpected due to the absence of 9'-*cis* forms of carotenoid in the natural, algal, diet. Amounts of lutein and isozeaxanthin are consistently higher in the female rather than male gonads with levels of these peaking shortly after spawning. The presence of 9'-*cis* echinenone as the major carotenoid contributing to the pigmentation/ colour of the gonad is an important observation in terms of developing artificial diets for urchin cultivation. The implications of this are discussed further.

EELGRASS RESPONDS TO OYSTERS AND GROW-OUT METHODS IN AN AQUACULTURE SETTING

Heather Tallis (University of Washington), Jennifer Ruesink (University of Washington), Brett Dumbauld (Hatfield Marine Science Center), Sally Hacker (Oregon State University), Lorena Wischart (Oregon State University)

Aquaculture has been shown to have negative impacts on eelgrass and certain aquaculture practices have been banned in the Pacific Northwest (USA) to protect eelgrass as valuable habitat. We argue that the magnitude of tradeoffs between aquaculture and biodiversity depends on the ecological details of the production system. We explored three aquaculture systems (dredged on-bottom, hand picked on-bottom, long lines) to quantify the tradeoffs between oyster (*Crassostrea gigas*) aquaculture and eelgrass (*Zostera marina*) habitat. Capitalizing on large scale "manipulations" for aquaculture in Willapa Bay, WA, we found lower eelgrass density in all aquaculture systems relative to uncultivated areas in 2002 – 2004. Dredged beds had the lowest eelgrass densities (~50% < uncultivated) while hand picked and long line beds were intermediate. After experimental dredging, eelgrass densities recovered in 1 - 4 years, depending on site and disturbance intensity. Additionally, eelgrass density declined with oyster density in cultivated beds, although oysters were sparsely planted (~20% cover). Eelgrass growth ($\text{g g}^{-1} \text{d}^{-1}$), measured in 2004, also varied by aquaculture type and oyster density, although patterns in this individual-level indicator were context-specific. Total above-ground production was largely driven by variation in eelgrass density,

so dredged beds had the lowest production. Our findings do not support regulations against all on-bottom aquaculture because hand picked beds showed the least tradeoff in terms of eelgrass production and were 1.5 - 2.3 times more productive than dredged beds. We suggest several management options that may allow the continued co-existence of aquaculture and eelgrass in the Pacific Northwest.

SETTLEMENT OF *CRASSOSTREA ARIAKENSIS* LARVAE UNDER CONDITIONS FOUND IN THE CHESAPEAKE BAY

Mario N. Tamburr i (Chesapeake Biological Laboratory / UMCES), Mark W. Luckenbach (Virginia Institute of Marine Science), Denise L. Breitburg (Smithsonian Environmental Research Center), Stephanie M. Bonniwell (Virginia Institute of Marine Science)

The Asian oyster (*Crassostrea ariakensis*) is being considered for introduction into the Chesapeake Bay. However, our current understanding of the biology and ecology of *C. ariakensis* is insufficient to predict whether an introduction will be successful, provide desired benefits, or have adverse impacts. Behavior of native oyster (*C. virginica*) pediveligers has been studied for many years and it is well established that they use a variety of habitat characteristics when selecting a site for colonization. Perhaps the most important of these are chemical cues emitted by adult conspecifics, which can lead to gregarious larval settlement and dense, persistent reef communities. Conversely, almost nothing is known about how larvae of *C. ariakensis* respond to conditions found in Chesapeake Bay or about the critical life history processes of settlement and metamorphosis.

We have examined how the behavior and substrate preference of two *C. ariakensis* strains (“south China” and “west coast”) at the time of settlement compares with that of *C. virginica*. Results demonstrate many similarities but also a few important differences. For example, both species and strains of larvae greatly prefer natural substrates (e.g. shell) covered with biofilms for colonization but the west coast strain of *C. ariakensis* exhibited greater attachment onto manmade substrates (e.g. fiberglass) than *C. virginica*. Waterborne chemical cues emitted by adult oysters were also found to enhance substrate attachment for all larval forms, whereas initial data suggests hypoxia inhibits larval attachment with the south China strain of *C. ariakensis* perhaps most sensitive to low oxygen conditions.

ASSESSMENT OF RED ABALONE, *HALIOTIS RUFESCENS*, POPULATIONS AT SAN MIGUEL ISLAND, CALIFORNIA

Ian K. Taniguch i (California Department of Fish & Game), Peter L. Haaker (California Department of Fish & Game)

Abstract. San Miguel Island contains the southernmost large population of red abalone, *Haliotis rufescens*, along the Pacific coast, but commercial and recreational abalone fishing was closed in 1997 because of a general decline. Since then, red abalone size frequency has improved at San Miguel Island and at some surrounding locations. The California Fish and Game Commission has recently adopted an Abalone Recovery and Management Plan which includes a provision for reopening a commercial and recreational fishery at San Miguel Island. To address the potential fishery, an assessment of the abalone resources will need to be completed.

Diving surveys, using 2 x 30 m band transects to collect density and size frequency data, are optimized using stratification of suitable abalone habitat at San Miguel Island within 1 nautical mile square (1 min x 1 min) grids. Maps of giant kelp, *Macrocystis pyrifera*, beds are used as a surrogate for hard benthic abalone habitat, and are overlain on the 1 mile square grids in a geographic information system. Within the common grid-kelp areas, thirty points are randomly selected for diving surveys.

Data will be used to determine red abalone size frequency and density for management, and compared to historic size frequency data. It will also provide comparisons between areas in and out side of recently established marine protected areas.

**EVIDENCE OF A RESPONSE TO UNINTENTIONAL SELECTION FOR FASTER DEVELOPMENT
ASSOCIATED WITH INBREEDING DEPRESSION IN *C. GIGAS***

Nicolas Taris (Ifremer - La Tremblade, France), Frederico M. Batista (INIAP/IPIMAR - Olhão, Portugal), Eric Marissal (Grainocéan - St Martin de Ré, France), Pierre Boudry (Ifremer - La Tremblade, France)

Direct and indirect consequences of selective breeding in marine bivalves still remain largely unexplored. For species with two-phase life cycles, like the Pacific oyster (*Crassostrea gigas*), most studies have focused on juvenile and adult stages, but relatively few have considered the larval stage, especially in a domestication context. We assessed the impact of hatchery practices on larval traits, notably on larval growth (due to the culling of slow growing larvae), by the study of larval progenies. Larvae originating from crosses using parental oysters both from natural beds and from hatchery broodstock which had been selected for adult growth and shell shape for seven generations. A set of three microsatellite loci was used to compare genetic variability in the two parental broodstocks and to establish the relatedness between pairs of individuals within each broodstock. The mean relatedness was nearly six times higher in the hatchery broodstock than in the wild broodstock. On one hand, our results show a lower mean survival associated with larger variation of growth rate showing a bimodal distribution for the hatchery larval population. On the other hand, a higher success at metamorphosis was observed for the surviving larvae bred from hatchery parents. The results suggest that some of these larvae exhibited inbreeding depression but this was balanced by an overall positive response to selective pressures for larval growth.

**EVALUATION OF SUSPENDED ADPI® BAGS VS. BOTTOM PLANTING FOR USE AS BAY SCALLOP
SPAWNER SANCTUARIES.**

Stephen T. Tettelbach (Southampton College of Long Island University), Andrew Weinstock (Southampton College of Long Island University), Dennis Bonal (Southampton College of Long Island University), Chelsea Fitzsimons-diaz (Southampton College of Long Island University), Richard Ames (Southampton College of Long Island University), Katherine Newman (Southampton College of Long Island University)

An off-bottom culture system for bay scallops (*Argopecten irradians irradians*), employing stacks of 15 mm mesh ADPI® bags (= arrays), was compared to bottom (free) planting as an alternate method for establishing spawner sanctuaries. Two stocks of hatchery-reared scallops were separately overwintered and then deployed at one site in Northwest Harbor, East Hampton, NY in ADPI arrays (50, 100, or 200 scallops/bag = 117, 234, or 468 scallops/m², respectively) or free-planted at a mean density of ~95 scallops/m² on 30 March-1 April 2005. In all, 12,600 scallops were stocked into ADPI arrays and ~235,000 scallops were free-planted. Overall survival, to late September 2005, of free-planted scallops (~31%) was better than that of scallops in ADPI bags (~18%). Cumulative mortality increased gradually in both scallop groups over the course of the study. Shell growth, from an initial mean stocking size of 38-40 mm, was significantly greater in free-planted scallops compared to those held in arrays ($t=7.10$; $p<.0001$). The timing of spawning, as determined via biweekly monitoring of gonad indexes, was similar in both groups of scallops. After 4.5 months, biomass of epifaunal fouling organisms on free-planted scallops (mean = 0.88 g whole wet weight per scallop) was lower than that on scallops held in ADPI bags (mean = 3.22 g whole wet weight per scallop). A second field season in 2006 will be used to evaluate a modified version of the initial ADPI array system.

VARIABILITY OF PHYTOPLANKTON BIOMASS IN THE DAMARISCOTTA RIVER ESTUARY

Brian Thompson (University of Maine), Mary Jane Perry (University of Maine), Christopher Davis (Pemaquid Oyster Company)

Phytoplankton biomass and environmental parameters in the Damariscotta River Estuary, Maine, USA, were quantified over space and time to better assess the carrying capacity of this prime location for shellfish mariculture. Water samples were collected 2 to 5 times a week between February and October 2005 at three stations in the estuary – mouth, middle, and head – and analyzed for chlorophyll *a* and pheopigment concentrations, dissolved inorganic nutrients, temperature, and salinity, with less frequent collection for phytoplankton taxonomic identification. In summer, additional locations were sampled from a small boat and with moorings. Stations between the middle and head of the estuary were occupied over two separate fortnightly tidal cycles between July

and September; moored instruments were deployed from late August to early September. Photosynthetically available radiation was continuously recorded at the middle station at the Darling Marine Center dock. An offshore mooring, GoMOOS Buoy E, provided additional data to assess external forcing on the estuary. In 2005, the late winter phytoplankton bloom at the middle station was delayed and significantly smaller in both magnitude and duration in comparison to blooms in the previous two winters. Nitrate concentrations were highest at the beginning and end of the year, with a significant draw down of all nutrients concurrent with the late winter bloom. Chlorophyll concentrations were typically highest at locations in the upper estuary. Identification of regions with persistent occurrence of high phytoplankton biomass throughout the growing season are important to oyster farmers for determining locations for culturing.

GENETIC AND ECOLOGICAL INTERACTIONS IN A MUSSEL HYBRID ZONE.

R.J. Thompson (Ocean Sciences Centre, Memorial University of Newfoundland), D.J. Innes (Ocean Sciences Centre, Memorial University of Newfoundland), J.B. Lowen (Ocean Sciences Centre, Memorial University of Newfoundland), M.B. Miranda (Ocean Sciences Centre, Memorial University of Newfoundland)

Understanding the processes that maintain the coexistence of closely related species requires studies of life-history variation, competition and hybridization. *Mytilus edulis* and *M. trossulus* coexist and hybridize throughout Atlantic Canada. Nuclear and mtDNA genetic markers have shown that F₁ hybrids are very rare (< 1% of the population) but individuals of mixed genotype (backcrosses) account for about 25%. Larvae and small mussels are predominantly *M. trossulus* or hybrids whereas larger mussels are almost exclusively *M. edulis*. A cohort analysis of laboratory-produced mussels of known species composition transplanted to the field showed that *M. edulis* grew faster and had a greater survival rate than *M. trossulus* or hybrids. A detailed analysis of energy allocation to soft tissues and shell demonstrated that *M. trossulus* had a greater investment in reproductive tissue and a lower investment in shell mass than *M. edulis*. Hybrids had an intermediate allocation pattern. A lower investment in shell mass, adductor muscle mass and byssus production resulted in a greater susceptibility of *M. trossulus* to crab and starfish predation. Life-history trade-offs involving reproduction, growth and survival are probably instrumental in explaining the coexistence of *M. edulis* and *M. trossulus*. Furthermore, a combination of reduced interspecific fertilization success (prezygotic) and post-fertilization mortality (postzygotic) results in partial reproductive isolation which can explain the observed frequency of hybrids.

MULTIVARIATE ANALYSIS OF SPATIAL VARIABILITY OF OYSTER-REEF COMMUNITIES: THE INFLUENCE OF SALINITY

S. Gregory Tolley (Florida Gulf Coast University, Coastal Watershed Institute), Aswani K. Volety (Florida Gulf Coast University, Coastal Watershed Institute), Mike Savarese (Florida Gulf Coast University, Coastal Watershed Institute), Christi M. Linardich (Florida Gulf Coast University, Coastal Watershed Institute), Laura D. Walls (Florida Gulf Coast University, Coastal Watershed Institute), Edwin M. Everham III (Florida Gulf Coast University, Coastal Watershed Institute)

When assessing oyster-reef habitat in estuaries, it is important to understand the contribution of salinity to the spatial variability of associated organisms. How comparable is community structure among stations located along the salinity gradient of an estuary or among tidal tributaries experiencing different levels of freshwater inflow? To address these questions, multivariate techniques were employed to analyze decapod crustacean and fish abundance data. Organisms were collected at three reefs along the salinity gradient (upper, middle, lower) of three estuaries: Caloosahatchee River, Estero River and Bay, and Faka Union Canal and Bay. Additional collections were made in Estero Bay from oyster reefs located at the mouths of five tidal tributaries. Cluster analysis did not reveal any natural groupings by estuary. Analysis of similarity did reveal significant differences among sites along the salinity gradients within estuaries and between Estero Bay tributaries. Multidimensional scaling identified community structure present at upper stations as distinct from that downstream, and at high-flow tributaries as distinct from that near low-flow tributaries. Upper stations and stations near high-flow tributaries were typified by the mud crab *Eurypanopeus depressus* and gobiid fishes. Downstream stations and stations near low-flow tributaries were typified by *E. depressus* and the porcelain crab *Petrolisthes armatus*. Percent dissimilarity was greatest when upper and lower stations were compared or when high-flow and low-flow stations were compared. Variability in multivariate

structure tended to be higher upstream or in association with high-flow tidal tributaries—a pattern suggesting that freshwater inflow is disturbing communities at these locations.

CAN HEAT SHOCK PROTEIN 70 EXPRESSION BE USED AS A BIOMARKER FOR ENVIRONMENTAL STRESS IN THE EASTERN OYSTER?

Nobuo Ueda (University of South Alabama), A.A. Boettcher (University of South Alabama)

Significant declines in eastern oyster, *Crassostrea virginica*, populations have occurred along the coasts of the eastern United States and Gulf of Mexico. Anthropogenic disturbances are thought to be the main cause of these declines, with changes in abiotic environmental conditions, either in conjunction with, or independent of these anthropogenic factors contributing to increased mortality in specific areas. Understanding the responses of oysters to environmental stressors and developing simple tools for detecting their effects is critical for the successful monitoring of oyster populations. As a part of the Alabama Oyster Reef Restoration Program, we have been examining the usefulness of heat shock protein 70 (HSP 70) expression as a biomarker of environmental stress in *C. virginica*. As has been shown with other oyster species, three isoforms of HSP 70, two constitutive (77 and 72 kDa) and one inducible (69 kDa), have been identified in the eastern oyster, *C. virginica*. However, it is not known how different environmental stresses impact the expression of these three isoforms. This study examined differences in expression of HSP70 isoforms in *C. virginica* based on both type of environmental stress (salinity, temperature, and dissolved oxygen) and age of the oyster. Levels of environmental stress, type of stress, and age of the oyster all impact HSP 70 expression, with differences in the expression levels among isoforms of HSP 70 associated with the different factors. Clarifying how these factors interact will allow for a better evaluation of the effectiveness of HSP 70 as a biomarker.

USING QUANTITATIVE PCR AND DNA BARCODES TO QUANTIFY AND IDENTIFY MARINE INVERTEBRATE LARVAE

Brent Vadopalas (University of Washington School of Aquatic and Fishery Sci), Joshua V. Bouma (University of Washington School of Aquatic and Fishery Sci), Chemine R. Jackels (University of Washington School of Aquatic and Fishery Sci), Carolyn S. Friedman (University of Washington School of Aquatic and Fishery Sci)

Information on the relationship between behavior and dispersion in marine invertebrate larvae has been hampered by the lack of suitably rapid techniques for identification and quantification from field samples. The need to understand larval dispersal dynamics of pinto abalone (*Haliotis kamtschatkana*), recently listed as a species of concern (U.S.) and threatened (Canada), motivated the development of a high-throughput method for identification and quantification of abalone larvae in seawater samples. Seawater was filtered through a 70 μ m screen and DNA was extracted from all organisms present in sample retentate. Species-specific Cytochrome Oxidase I (COI) mitochondrial DNA sequence was used as template for Quantitative PCRs (QPCR) containing primers and a dual-labeled hydrolysis probe. Direct enumeration of larvae using light microscopy was used to verify quantity estimates derived from QPCR standard curves. To avoid bias from either underestimation of quantity or false negatives, abalone-specific reactions were multiplexed with primers, probe and exogenous template as an internal positive control (IPC) to indicate partial or full PCR inhibition. Differences between direct counts ($n = 1-141$) and QPCR estimates were not significant, and the average coefficient of variation was 0.56 for within-run replicates, indicative of high accuracy and precision. We estimate that 80 samples can be processed from DNA extraction through QPCR in about four hours at a cost of approximately \$2/ sample. Our results demonstrate that QPCR may have high utility for high throughput identification and quantification of specific marine invertebrate larvae in seawater samples via counts of mtDNA COI “barcodes” using constructed standard curves.

PROTANDRY, MATURATION, AND SPAWNING IN CULTURED INTERTIDAL GEODUCK (PANOPEA ABRUPTA) CLAMS

Brent Vadopalas (University of Washington School of Aquatic and Fishery Sci), Cyrus S.Y. Ma (University of Washington School of Aquatic and Fishery Sci), Jonathan P. Davis (Baywater, Inc.), Carolyn S. Friedman (University of Washington School of Aquatic and Fishery Sci)

Among the challenges facing aquaculture of endemic species are potential negative effects of gene flow from cultured to wild populations. Solutions include maturation control, monosex outplants, spatial or temporal separation, and harvest before sexual maturation. To assess whether cultured intertidal geoduck clams mature and spawn before harvest, we sampled 12 geoduck ten times over the course of one year (2004-2005) from each of five year classes (1999-2003) planted conterminously in south Puget Sound, Washington. Additional sampling occurred through a second spawning season from the 2001, 2002, and 2003 year classes. Individuals were sexed, weighed, and measured; histological sections of gonad were classified by developmental stage (early active, late active, ripe, partially spawned, spent). Using image analysis, we calculated a gonadal-somatic index for each individual. Of geoducks age 1.5-2.2 years, approximately 23% exhibited evidence of spawning and 71% were mature, compared to 68% and 89% respectively in geoducks age 4.5-5.0 years. We estimate that 50% maturation occurs at 64 mm shell length at this site. Male:female ratios in 2-5 year old geoducks were male-biased relative to the predominate 1:1 sex ratios observed in wild populations ($p \ll 0.05$), providing evidence of facultative protandric dieoccy. Maturation stage in cultured geoducks was temporally correlated with maturation stage in wild populations, indicating no temporal separation. Taken together, these data suggest that (1) pre-maturation harvest to prevent gene flow may be untenable and (2) future efforts be directed toward exploring the viability of maturation control in this species.

POPULATION STRUCTURE IN THE EASTERN OYSTER *CRASSOSTREA VIRGINICA* ASSESSED BY SINGLE NUCLEOTIDE POLYMORPHISMS

Robin L. Varney (University of Delaware), Patrick Gaffney (University of Delaware)

Single nucleotide polymorphisms (SNPs) are abundantly distributed throughout plant and animal genomes. Because the number of loci is virtually unlimited and they are amenable to robust high-throughput genotyping, SNPs are rapidly becoming standard tools for the analysis of population structure, genetic mapping, and taxonomic identification. Our goal is to evaluate population structure of the eastern oyster throughout its range from Atlantic Canada to the Yucatan Peninsula, using a large number of SNPs identified in known genes (Type I SNPs). While previous work has shown a clear genetic break between Atlantic and Gulf coast populations in mitochondrial DNA, studies of geographic variation in nuclear loci (allozymes and anonymous DNA markers) have yielded mixed results. Existing genomic and expressed sequence tag (EST) databases of *C. virginica* have been mined for the development of both anonymous and type I markers, with recent efforts focused on genes thought to be involved in disease response. Direct sequencing of amplified targets has revealed extensive polymorphism in *C. virginica*. We have developed a set of nuclear SNP markers to examine the genetic diversity of *C. virginica* throughout the species range and provide multiple markers for genetic linkage mapping. Significant heterogeneity in allelic frequencies among the geographic locations was observed for each locus. Our results are consistent with the Gulf-Atlantic split previously observed with other markers. In addition, clinal patterns in allelic frequencies in Atlantic populations are suggested for some loci.

ASSESSING IMPACTS OF SHELLFISH AQUACULTURE ON EELGRASS POPULATIONS IN EASTERN LONG ISLAND SOUND

Jamie Vaudrey (University of Connecticut), Tessa Getchis (University of Connecticut - Sea Grant Extension Program), Bob Britton (none)

Eelgrass (*Zostera marina*) is the dominant vascular plant of northern estuaries of the east and west coasts of the United States. Eelgrass beds provide critical ecological functions such as removing nutrients and stabilizing fine sediments. Beds also provide critical habitat to a myriad of marine organisms including juvenile fish, shellfish, and crustaceans, among others. Eelgrass is on the decline in most estuaries, coincident with urbanization and the resulting increase in nutrient loads to the coastal waters. The presence of eelgrass is considered an indicator of a "healthy" system and much attention has been focused on investigating potential causes of the loss of eelgrass and determining methods to minimize the decline in eelgrass abundance.

Bivalve aquaculture, specifically the utilization of submerged cultivation and depuration gear such as cages, has been implicated as a potential source of negative impacts to eelgrass populations. However, shellfish aquaculture

gear has also been shown to provide an equivalent or greater degree of ecosystem services as submerged aquatic vegetation such as eelgrass.

This study was conducted to determine the type and degree of impacts and benefits that oyster bottom cages have on eelgrass and surrounding water and sediment quality, in order to provide producers and policy-makers with the scientific data needed to make informed decisions regarding the siting of aquaculture in Long Island Sound.

Preliminary results suggest significant differences in eelgrass growth and sediment quality among treatments.

FRESHWATER MUSSELS: SPECIES ROLES, ECOSYSTEM SERVICES AND CONSERVATION.

Caryn C. Vaughn (University of Oklahoma), Daniel E. Spooner (University of Oklahoma), Heather S. Galbraith (University of Oklahoma)

Our lab is using an integrative approach of comparative field observations, field experiments, and laboratory experiments to address three fundamental questions concerning freshwater mussel guild: (1) What is the importance of the mussel guild to stream ecosystem function?; (2) Do species perform different ecological roles?; and (3) Do species roles vary with environmental context? Our results indicate that many ecosystem services performed by mussels (algal clearance, nutrient excretion, biodeposition) are linearly related to community biomass; thus, there is the potential for strong ecosystem effects when mussel biomass is high and hydrologic residence times are long. Algal growth is higher on the shells of living mussels compared to shells alone, and macroinvertebrate richness and densities are higher on the shells of living mussels and in mussel patches than in other streambed areas. Field and laboratory experiments demonstrate strong effects of a potential driver species, *Actinonaias ligamentina*, on factors such as periphyton biomass, but comparatively weak diversity effects. In addition, species effects are context-dependent and regulated by abiotic factors such as temperature and flow. Our results indicate that some mussel species are performing differently in streams and are thus not redundant, but that performance and potential redundancy are context dependent. Using an extensive dataset on mussel community composition and biomass from across southeastern Oklahoma and western Arkansas, we are scaling up our results to make predictions about mussel contributions to ecosystem services in natural streams and how this may vary as the environment changes.

MANAGING A DUNGENESS CRAB (*CANCER MAGISTER*) FISHERY FOR MULTIPLE USER GROUPS: THE PUGET SOUND, WASHINGTON, EXPERIENCE

Lisa Veneroso (Washington Department of Fish and Wildlife), Richard Childers (Washington Department of Fish and Wildlife)

Intensive fishery management strategies have been adopted for Dungeness crab (*Cancer magister*) in Puget Sound, Washington, in response to increases in fishing pressure. In 1994, a U. S. District Court decision ruled treaty Indian tribes in Washington State have the legal right to harvest up to 50% of shellfish, including Dungeness crab, in their usual and accustomed fishing grounds. Prior to 1995, Dungeness crab were harvested exclusively by a state commercial and recreational fishery using a passive "3S" harvest strategy of minimum size, set season, and sex (male crab only). The guiding assumption of the 3S strategy being that male crab $\geq 6\frac{1}{4}$ inches are surplus and can be harvested at a high rate and not threaten reproductive success or the sustainability of the resource. Since 1995, crab harvest has increased from three million to eight million pounds, harvest seasons have been adjusted on a regional basis to more closely adhere to molt timing, and annual pre-season quotas have been established. Currently, the fishery is managed through harvest sharing agreements that allocate harvest between 15 treaty tribes conducting predominantly commercial fisheries, a state commercial fishery consisting of 250 licenses and a recreational fishery with an estimated 200,000 anglers. Fishery managers are questioning whether the management scheme adopted for Puget Sound is sustainable considering the high exploitation rate, the handling mortality of female and sub-legal size male, and the lack of stock abundance information needed to evaluate the appropriateness of the pre-season quotas.

ENERGY STORAGE AND UTILIZATION IN RELATION TO GAMETOGENESIS OF *ARGOPECTEN VENTRICOSUS*

Janzel Rogelio Villalaz Guerra (Departamento de Biología Marina, University of Panama), Juan A. Gomez Herrera (Departamento de Biología Marina, University of Panama), Luis D'Croz (Departamento de Biología Marina, University of Panama)

A laboratory study was carried out to observe changes in reproduction of *Argopecten ventricosus* by using histological techniques in gonads and chemical analysis of digestive gland, adductor muscle, mantle-gills and gonad. These data allowed us to calculate energy storage related to gametogenesis of *A. ventricosus*. During 66 days, combinations of monocultures (50:50) of C-ISO and CH-1 were added daily to a tank with filtered and aerated seawater. Salinity and temperature of the water were measured with a salinity-temperature probe meter (YSI). Phytoplankton densities were recorded by direct count with a hemacytometer. This study is a contribution to the reproductive biology of *A. ventricosus* and fisheries management of the tropical scallop.

COMPARISON OF IMMUNE PARAMETERS BETWEEN OYSTER STOCKS AND SPECIES WITH DIFFERENT SUSCEPTIBILITY TO INFECTION BY *BONAMIA OSTREAE*

Antonio Villalba (Centro de Investigacións Mariñas, Xunta de Galicia, Spain), Pilar Comesaña (Centro de Investigacións Mariñas, Xunta de Galicia, Spain), Sandra M Casas (Centro de Investigacións Mariñas, Xunta de Galicia, Spain), Asunción Cao (Centro de Investigacións Mariñas, Xunta de Galicia, Spain)

The parasitic protozoan *Bonamia ostreae* causes oyster *Ostrea edulis* mass mortalities throughout the European Atlantic coast, whereas the allochthonous oyster *Crassostrea gigas* is resistant to bonamiosis. Previous studies indicated that the *O. edulis* strain "Rossmore" produced through selective breeding has increased tolerance to bonamiosis compared to other European stocks of flat oyster. The immune capability of oyster *O. edulis* stocks with different susceptibility to bonamiosis and that of oysters *Crassostrea gigas* were compared to assess whether some immune parameters could explain the differences in susceptibility to infection by *Bonamia ostreae* between oyster stocks/species. The *O. edulis* stocks used in the comparisons were: Rossmore (selected strain), oysters from Tralee Bay (Ireland) where the parasite had not previously been detected, and oysters from natural beds in Galician Rías (Spain) that are affected by bonamiosis. The immune parameters selected for comparisons were total haemocyte count and differential haemocyte count of the haemolymph; phagocytic ability of haemocytes; intra- and extracellular production of superoxide anion, production of hydrogen peroxide and production of nitric oxide by haemocytes; antibacterial activity in haemocytes and plasma; phenoloxidase and acid phosphatase activities in haemocytes and plasma; and protease inhibitory activity in plasma. Consistent differences in any of the immune parameters studied between *O. edulis* stocks that explained differences in susceptibility to bonamiosis were not found. However, some of the immune parameters would be candidates to explain differences in susceptibility to bonamiosis between *O. edulis* and *C. gigas*.

DIFFERENT TYPES OF NEOPLASIA IN *CRASSOSTREA GIGAS*, *VENUS VERRUCOSA* AND *MYTILUS GALLOPROVINCIALIS* FROM THE SPANISH COAST

Antonio Villalba (Centro de Investigacións Mariñas, Xunta de Galicia, Spain)

New cases of neoplasia corresponding to three types of neoplastic condition are described. One out of 22 oysters *C. gigas* taken from an oyster farming area in the Ría de Arousa (Galicia, NW Spain) showed disseminated neoplasia (DN). Histological analysis revealed abundant abnormal cells with large (up to 5 µm) round nucleus and scant cytoplasm infiltrating the connective tissue close to the stomach and in blood sinuses, suggesting invasiveness; mitotic figures were frequent but other organs appeared free of abnormal cells, suggesting an early stage of disease. DN prevalence in oysters *Ostrea edulis* cultured in that area is high, thus the possibility of interspecies transmission should be assessed. One out of 25 warty clams *Venus verrucosa* taken from a bed close to Maó (Balearic Islands, Spain) appeared affected by another type of neoplasia. Histological analysis revealed abnormal cells with large, extremely elongated (up to 10 µm long) nucleus and scant cytoplasm replacing normal cells of the connective tissue of the visceral mass; mitotic figures were frequent and no abnormal cell was observed in blood vessels. One out of thousands of mussels *Mytilus galloprovincialis* examined in a histopathological programme ran for 10 years throughout the Galician Rías was affected by germinoma. The mussel was hermaphrodite, with some gonad follicles containing either male or female gametes and some other follicles appeared filled with highly basophilic cells, tightly packed, likely deriving from germinal epithelium of the follicles. Mitotic figures were seen in those masses but they were not frequent.

STOCK-RECRUIT RELATIONSHIPS FOR CRASSOSTREA VIRGINICA AND C. ARIAKENSIS IN CHESAPEAKE BAY DEVELOPED FOR A DEMOGRAPHIC OYSTER POP

Jon H. Vølstad (Versar), Jodi R. Dew (Versar)

A demographic population model is being developed to support an ecological risk assessment (ERA) of the proposed introduction of *Crassostrea ariakensis* and restoration alternatives of *C. virginica* in Chesapeake Bay by the states of Maryland and Virginia. As part of the model, a stock-recruit relationship was developed and is defined as the ratio of number of spat recruited to October (when the annual Fall survey is performed by the Maryland Department of Natural Resources (DNR)) to number of standardized (to 77mm) female oysters. Stock-recruit relationships were estimated using empirical data collected from 1980 to 2004 by the DNR in annual surveys of oyster beds in Maryland, shell length data, sex ratio by size estimates, and fecundity relationships between shell length and number of eggs produced.

R-square value for *C. virginica* stock-recruit relationship was 0.45 with 2.6 spat produced per standardized female oyster. The number of spat produced was 2.6, 2.8, and 2.1 per standardized female oyster when the stock-recruit relationship was examined per average, dry, and wet rainfall year, respectively. This stock-recruit relationship for *C. virginica* is used for *C. ariakensis* to predict the number of spat produced per standardized *C. ariakensis* stock to a 77mm female *C. virginica* oysters, under the assumption of similar mortality rates for spat of both species to October. The stock-recruit relationship for both species can also be altered to include gamete loss from inviable hybrids in cross-fertilization when species are reproducing in close proximity to each other.

ESTIMATION OF ANNUAL MORTALITY RATES FOR EASTERN OYSTERS (CRASSOSTREA VIRGINICA) IN CHESAPEAKE BAY BASED ON BOX COUNTS AND APPLI

Jon H. Vølstad (Versar), Jodi R. Dew (Versar), Mitchell Tarnowski (Maryland Department of Natural Resources)

In an effort to restore the ecological role of oysters in Chesapeake Bay and the economic benefits of a commercial fishery, the states of Maryland and Virginia are considering the introduction of the non-native Asian oyster, *Crassostrea ariakensis*. As part of the ecological risk assessment (ERA) to evaluate the proposed action and restoration alternatives, demographic modeling is applied to project the change in populations of both Asian and eastern oyster populations in the Bay in space and time. We present two approaches to parameterize the annual mortality rates for the Asian and eastern oyster for the demographic model. Mortality rates were estimated from empirical data collected by the Maryland Department of Natural Resources (DNR) in annual surveys of oyster beds in Maryland. We compared counts of recent boxes (dead oysters including gapers about 2 weeks old, in which tissue is still found within the shell, as well as boxes with no fouling or sedimentation on the inner valve surfaces), old boxes (dead oysters in which fouling and/or sedimentation is found on the inner valve surfaces and no tissue remains), and live oysters in both market and small size classes. Our mortality estimates from recent box counts consistently differentiated between years with high versus low disease intensity and wet and dry years, and also between salinity zones. In contrast, traditional estimates of yearly mortality based on total box counts were often out of phase with measured disease intensity levels, and type of weather year (dry or wet).

UNDERSTANDING DIET DEVELOPMENT IN RELATIONSHIP TO SEA URCHIN GAMETOGENESIS

Charles W. Walker (The University of New Hampshire), S. Anne Boettger (The University of New Hampshire)

One challenge when culturing adult green sea urchins is the formulation of diets yielding palatable and marketable roe. Current commercial diet formulations vary and yield taste, color and texture variation of the product. High protein content of diets related to production of large gonads yields poor taste, while color is related to carotenoid content. Nutrient uptake into nutritive phagocytes (NPs) of the gonad (somatic cells in both sexes) is an important factor as sea urchin gonads are large before and during gametogenesis due to nutrient storage by NPs. Initially, NPs incorporate nutrients into membrane-bound vesicles in the cytoplasm and mobilize them as germ cells enlarge during gametogenesis. Mechanisms of nutrient delivery to NPs and from them to germ cells have been a focus of recent studies. At completion of oogenesis NPs contain basal amitotic oogonia and small/intermediate-sized

vitellogenic primary oocytes, though their major activity is enclosure and nourishment of vitellogenic oocytes prior to maturation and release. Mechanisms regulating differential nutrient release by sea urchin NPs and their selective uptake by germ cells at different stages of gametogenesis are unknown though there is coordination between NPs and germ cells. Oocytes must be receptive to incorporate nutrients released by NPs at specific times. Endocytosis by oocytes increases two fold, uptake of MYP increases 10-fold during this time. It is inevitable that changes in nutrients delivered to germ cells may be related to the quantity/quality or kind of food ingested by sea urchins, which has to be taken into account during diet development.

PHYSIOLOGICAL RESPONSES TO SALINITY STRESS IN THE FLATBACK MUD CRAB *EURYPANOPEUS DEPRESSUS*

Laura D. Walls (Florida Gulf Coast University, Coastal Watershed Institute), S. Gregory Tolley (Florida Gulf Coast University, Coastal Watershed Institute)

Estuaries in Southwest Florida experience highly altered freshwater inflow resulting in part from anthropogenic activities. To gauge possible effects of altered salinity regimes on species in these systems, physiological responses to salinity stress were investigated in the flatback mud crab *Eurypanopeus depressus*, a dominant member of oyster-reef communities in the region. Haemolymph osmolality and oxygen consumption were measured for animals acclimated to 5‰, 15‰, 25‰, and 35‰ over a period of four weeks in a laboratory setting. Haemolymph osmolality varied significantly with respect to salinity, with measured osmolalities at each salinity treatment being significantly different ($p < 0.0001$) from one another. Oxygen consumption also varied significantly with salinity, with values recorded for the lowest salinity treatment (5‰) being generally higher ($p < 0.0001$) than all other treatments over the length of the study. Oxygen consumption did not vary over the first three weeks of the study for animals acclimated to 5‰ but declined significantly by week four. This general pattern of increased oxygen consumption in response to dilute salinity levels is a trend that has been documented previously in several estuarine species. The results of this study suggest that osmoregulation requires greater energy expenditure by *Eurypanopeus depressus* at subnormal salinity levels. Having to expend additional energy to meet osmoregulatory demands can result in reduced amounts of energy available for processes such as growth and reproduction, thereby affecting organism health. Suboptimal salinity levels resulting from altered freshwater inflow can thus significantly affect species abundance and distribution, specifically in the upper reaches of estuaries.

IMPACT OF WATER MOTION ASSOCIATED WITH RECREATIONAL BOATING AND HURRICANES ON INTERTIDAL OYSTER REEFS

Linda Walters (University of Central Florida), Paul Sacks (University of Central Florida), Loren Coen (South Carolina Department of Natural Resources), M. Yvonne Bobo (South Carolina Department of Natural Resources), Donnia Richardson (South Carolina Department of Natural Resources), Melinda Donnelly (University of Central Florida), Sarah Johnson (University of Central Florida), Heidi Deutsch (University of Central Florida)

In major recreational boating channels in shallow-water estuaries along the east coast of Florida, piles of disarticulated shells that extend up to 1 m above the high tide line on the seaward edges of intertidal reefs of *Crassostrea virginica* are becoming more and more abundant. As part of a larger study to understand the impact of these “dead margins” on critical habitats, we addressed the following questions: 1) what is the relative importance of boat wakes versus larger-scale, naturally generated (e.g. hurricane) wakes in shaping reef profiles, 2) what is the prevalence and intensity of oysters with *Perkinsus marinus* on pristine reefs versus reefs with dead margins, and 3) what is the potential for dead margins to be colonized by native and invasive plants. We documented significant shell movement from a single boat pass by a reef, while 22 m/s winds moved less than 1% of deployed shells. Additionally, the three hurricanes in 2004 impacting this area had negligible effects on reef profiles. Likewise, no significant differences were detected for *Perkinsus* in oysters between pre- and post hurricane sampling on dead or healthy reef margins. Red mangroves produce seeds year-round in central Florida and these seeds colonized dead margins if they recruited between winter and mid-summer. Water levels were higher each fall, preventing recruitment of red or late-summer black mangrove seeds at this time. Invasive Brazilian pepper seeds dispersed

each winter. Although these seeds were frequently observed on dead margins, none were retained long enough for recruitment to occur.

TO PIT OR NOT TO PIT?: COMPARISON OF OVERWINTERING METHODS FOR OYSTERS

William C. Walton (Cape Cod Coop. Ext. & Woods Hole Sea Grant), Ben Wright (Cape Cod AmeriCorps), Diane C. Murphy (Cape Cod Coop. Ext.)

The threat of ice damage has forced intertidal oyster farmers to devise strategies to reduce potential losses. In New England, some oyster farmers move their oysters from the farm location to storage area, or 'seed pit', that is typically cold (0-4° C) and damp (over 90% relative humidity). Qualitatively, local farmers have reported survival rates of over 90%.

We quantified survival and typical time to revival of oyster, (*Crassostrea virginica*) seed as a function of storage time. While survival was good and did not seem to vary over the course of the experiment, there was a clear step-wise drop in time to revival. Furthermore, a study of survival of seed held in the pits of local farmers suggested survival remained high ($\geq 86\%$) for seed held for ~4 months but dropped to 82% for those held an additional month. Furthermore, evidence suggests that second year seed do not survive pitting as well as first year seed.

Alternatives to pitting include leaving the oysters on the farm location or moving them to deeper water, below any ice that might form. We compared overwintering survival and growth of these three methods, both immediately post-winter and across the 2005 growing season. The lowest survival was in the treatment left on the flats, apparently due to damage from ice, while little to no growth was observed immediately post-winter. More surprisingly, there was a tendency for growth across the season to be slowest in the treatment moved to deeper water, relative to the other two treatments.

DISTRIBUTION OF CRASSOSTREA ARIAKENSIS IN CHINA

Haiyan Wang (Haskin Shellfish Research Laboratory, Rutgers University), Lumin Qian (Third Institute of Oceanology, Oceanic Administration, PRC), Guofan Zhang (Institute of Oceanology, Chinese Academy of Sciences, PRC), Xiao Liu (Institute of Oceanology, Chinese Academy of Sciences, PRC), Aimin Wang (Ocean College, Hainan University), Yaohua Shi (Ocean College, Hainan University), Nianzhi Jiao (Center for Marine Environmental Sciences, Xiamen University), Ximing Guo (Haskin Shellfish Research Laboratory, Rutgers University)

It is commonly assumed that *Crassostrea ariakensis* is synonymous with *C. rivularis* that, according to the literature, is abundant and widely distributed in China. However, at least three species, *C. gigas*, *C. hongkongensis* and *C. ariakensis*, have been reported as *C. rivularis* in China, creating uncertainties about the distribution of *C. ariakensis*. To determine the distribution of true *C. ariakensis* in China, we collected and classified 2,624 oysters from 50 locations along China's coast using species-specific DNA markers. *C. ariakensis* was found at 10 sites ranging from northern Shandong to Guangxi. While *C. ariakensis* had a wide geographical distribution, its occurrence within its range is patchy or scarce. Overall, *C. ariakensis* accounted for only 9.5% of all oysters collected. Large *C. ariakensis* populations were found in only three areas: Jiulong River in Fujian, Dongzao Harbor near Yangtze River and Yellow River basin in Bohai Sea, with none observed in-between. In Guangxi and Guangdong, *C. ariakensis* was present in all samples collected at low frequencies (0.5 – 17.5%). All three major populations are found in or near large rivers, and the absence of rivers may be a factor contributing to the fragmented distribution. At all sites, *C. ariakensis* co-existed with other species: *C. gigas* in Bohai Sea; *C. sikamea* at Dongzao Harbor; and *C. hongkongensis*, *C. angulata*, *C. sikamea* and *Saccostrea* species in southern China. *C. ariakensis* tended to occur subtidally, while other species were often found intertidally.

IDENTIFICATION OF CRASSOSTREA SPECIES FROM CHINA USING SNP-BASED MARKERS

Haiyan Wang (Haskin Shellfish Research Laboratory, Rutgers University), Ximing Guo (Haskin Shellfish Research Laboratory, Rutgers University)

China is home to 17 species of oysters and among them, five *Crassostrea* species, *C. hongkongensis*, *C. angulata*, *C. gigas*, *C. sikamea* and *C. ariakensis* are most common and commercially important. These five species often co-exist in the same estuary, and their identification using morphological characteristics is problematic. Genetic markers are needed for rapid and reliable identification of these oysters. Single nucleotide polymorphisms (SNPs) are simple and powerful markers for various genetic analyses. In this study, we developed species-specific SNP markers for the identification of common oysters from China. The mitochondrial cytochrome oxidase I (COI) gene and the nuclear 28S ribosomal RNA gene were used for marker development. DNA sequences from different species were either obtained by direct sequencing or downloaded from GenBank. Sequences were aligned, and species- and genus-specific SNPs were identified. Primers were designed for species/allele-specific amplification to generate fragments of different sizes in each species. A multiplex set of species-specific markers from COI was able to distinguish all five *Crassostrea* species in a single-tube PCR. It also separated *Ostrea* and *Saccostrea* species from *Crassostrea* species with the exception of *C. virginica* and *C. rhizophorae*. The 28S primer set was able to separate *C. hongkongensis*, *C. ariakensis* from other species, as well as *Saccostrea* and *Ostrea* species from *Crassostrea* species (except *C. virginica* and *C. rhizophorae*). The SNP-based markers do not require fluorescence-labeling or post-PCR digestion, providing a simple, fast and reliable method for oyster identification.

LOSS OF ALLELE DIVERSITY IN AN INTRODUCED POPULATION OF THE HERMAPHRODITIC BAY SCALLOP *ARGOPECTEN IRRADIANS*

Lingling Wang (Haskin Shellfish Res Lab, Rutgers, US), Huan Zhang (Institute of Oceanology, CAS, China), Linsheng Song (Institute of Oceanology, CAS, China), Ximing Guo (Haskin Shellfish Res Lab, Rutgers, US)

The bay scallop *Argopecten irradians*, a hermaphroditic bivalve native to the Atlantic coast of the United States, was introduced to China for aquaculture production in 1982. Now bay scallops dominate scallop farming in China and account for more than half of all scallop production. Bay scallop aquaculture is exclusively hatchery based and as the initial introduction consisted of only 27 scallops, there have been concerns about inbreeding and inbreeding depression in cultured populations in China. In this study, we used 11 simple sequence repeat (SSR) markers to compare genetic variation in cultured populations from China with that in a natural population from America. Eight of the 11 loci were polymorphic in all populations studied. Although the difference in heterozygosity was small, the Chinese populations lost 10 of the 45 alleles (22%) found in the wild population. The reduced allele diversity suggests that the Chinese populations experienced a severe bottleneck in genetic diversity during the introduction and subsequent hatchery production. Interestingly, the loss of rare alleles did not result in significant changes in heterozygosity. Fixation index of the Chinese populations were actually lower than that of the wild population, suggesting that the culture Chinese populations may represent a mixture of scallops from different introductions or populations. Effects of the reduced allele diversity are not clear at this time, but results of this study highlight the need for genetic monitoring and selective breeding in order to ensure the genetic health of introduced populations.

IDENTIFICATION OF *CRASSOSTREA ARIAKENSIS* USING ITS LENGTH POLYMORPHISM

Yongping Wang (Haskin Shellfish Research Laboratory, Institute of Marine an), Ximing Guo (Haskin Shellfish Research Laboratory, Institute of Marine an)

Oysters cannot be reliably identified using morphological characteristics alone. In an effort to develop genetic markers for oyster identification, we studied length polymorphism in internal transcribed spacers (ITS) between ribosomal RNA genes in 12 common species of Ostreidae: *Crassostrea virginica*, *C. rhizophorae*, *C. gigas*, *C. angulata*, *C. sikamea*, *C. ariakensis*, *C. hongkongensis*, *Saccostrea echinata*, *S. glomerata*, *Ostrea angasi*, *O. edulis*, and *O. conchaphila*. We downloaded and aligned ribosomal RNA sequences from all oyster and some other bivalve species to identify conserved sequences flanking ITS1 and ITS2. We designed two pairs of primers and optimized PCR conditions for simultaneously amplification of ITS1 and ITS2 in a single tube. Amplification was successful in all 12 species, and PCR products were visualized on high-resolution agarose gels. ITS2 was longer than ITS1 in all *Crassostrea* and *Saccostrea* species, while they were about the same size in three *Ostrea* species. No intraspecific



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variation in ITS length was detected. Among species, the length of ITS1 and ITS2 was polymorphic and provided unique identification of eight species or species pairs: *C. ariakensis*, *C. hongkongensis*, *C. sikamea*, *O. conchaphila*, *C. virginica*/*C. rhizophorae*, *C. gigas*/*C. angulata*, *S. echinata*/*S. glomerata*, and *O. angasi*/*O. edulis*. Two species within a pair were not distinguishable by ITS length. The ITS assay provides simple, rapid and effective identification of *C. ariakensis* and several other oyster species. Because the primer sequences are conserved, the ITS assay may be useful in the identification of other bivalve species.

**DEVELOPMENT AND CHARACTERIZATION OF EST-SSR MARKERS IN THE EASTERN OYSTER
CRASSOSTREA VIRGINICA**

Yongping Wang (Rutgers University), Ximing Guo (Rutgers University)

Simple sequence repeat (SSR) markers were developed from expressed sequence tags (ESTs) in the eastern oyster (*Crassostrea virginica*). ESTs of the eastern oyster were downloaded from GenBank and screened for SSRs that contained at least eight units of di-nucleotide repeats or five units of tri-, tetra-, penta- and hexa-nucleotide repeats. The screening of 9101 ESTs identified 127 (1.4%) SSR-containing sequences. Primers were designed for 88 SSR-containing ESTs with good and sufficient flanking sequences. PCR amplification was successful for 71 (81%) of the primer pairs including 19 pairs amplified fragments that were significantly longer than the expected size, probably due to introns. Sixty-six pairs that produced fragments size shorter than 800 bp were screened for polymorphism in five oysters from three populations using polyacrylamide gels, and 53 of them (80%) were polymorphic. Forty-five SSRs were labeled and genotyped in 30 oysters from three populations using an automated sequencer. Five of the SSRs amplified more than two fragments per oyster, suggesting that they belonged to duplicated loci, but they can still be used for segregation analysis. The remaining 40 SSRs had two alleles per individual including seven with null-alleles. In the 30 oysters analyzed, the SSRs had an average of 9.6 alleles per locus, ranging from 2 to 24. All 45 markers were used for segregation analysis in a family with 34 progeny. None of the 45 loci showed significant deviation from Mendelian ratios. This study demonstrates that ESTs are valuable resources for the development of genetic markers in the eastern oyster.

DEVELOPMENT OF FORMULATED FEEDS IN ECHINOID

Stephen A. Watts (University of Alabama at Birmingham), A.L. Lawrence (Texas A&M University System), M.L. Powell (University of Alabama at Birmingham), J.M. Lawrence (University of South Florida)

Successful sea urchin aquaculture will ultimately depend on high quality, nutritionally-complete, commercially-available feeds. We are developing practical, semi-purified and purified feeds that promote high rates of growth and survival. These feeds require the use of high quality ingredients that can be obtained from reputable commercial vendors. Semi-purified diets can be used to determine dietary requirements and/or toxicities of proximate nutrients in urchins. Purified diets can be used to evaluate requirements for micronutrients, including vitamins and minerals. We are currently evaluating semi-purified, cold-extruded feeds that range from 17 to 41 % protein, 15 to 36% carbohydrate, 4 to 12% crude fat (marine and non-marine sources), 1 to 5 % crude fiber, and 6 to 50% ash. These nutrients are evaluated using both purified and non-purified sources, both in combination and as the sole source. Amino acid profiles have been calculated to ensure adequate availability of indispensable amino acids. Fatty acid profiles have been determined to evaluate requirements for PUFA or HUFA. Data indicate that specific minerals and vitamins are required for normal physiological function. High production of gonads with marketable color and flavor is now possible. Empirically-derived levels of experimental nutrients are necessary as calculated levels may inaccurately estimate actual levels in the feeds. Supported by the Mississippi-Alabama Sea Grant Consortium.

NUTRITION RESEARCH IN ECHINOID: CONSIDERATIONS FOR METHODOLOGY

Stephen A. Watts (University of Alabama at Birmingham), M.L. Powell (University of Alabama at Birmingham), A.L. Lawrence (Texas A&M University System), J.M. Lawrence (University of South Florida)

Recent interest in commercial echinoid culture has led to studies of the nutritional requirements for many sea urchin species. As sea urchins are benthic, eurythermal, stenohaline and, in some cases, rheophilic organisms which feed at relatively slow or irregular rates, consideration of abiotic and biotic factors that affect feeding and growth



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characteristics can enhance our ability to evaluate nutritional requirements. We are attempting to standardize protocols for nutritional studies in echinoids. Considerations include standardized temperatures (species-specific), salinity, photoperiod, oxygen levels, water quality (including nitrogen levels, pH, hardness and alkalinity, natural vs. artificial seawater), flow rates, and *in situ* bacterial and fungal populations. System design (flow-through or recirculation) must be coupled with appropriate statistical design and evaluation (individual observations vs. group trials). Understanding life-history traits, including behaviors, pathologies, and natural growth variability, is essential. Feeds can include live or preserved natural food and practical, semi-purified and purified feeds. However, evaluation of natural food is of limited value in determining requirements because of lack of information about their content. Formulated (*not* artificial) feeds should be composed of quality ingredients available from commercial sources, whether practical, semi-purified or purified. Formulated feeds must have defined size, shape, texture, stability, and attractability, with defined handling and storage characteristics. Whenever possible, nutrient content should be determined empirically. Feed management strategies must be well defined, including feeding rates and rations. Finally, echinoid nutritional studies should use standardized metrics and responses for cross-species and cross-phylum comparisons. Supported by Mississippi-Alabama Sea Grant Consortium.

GROWTH OF BAY SCALLOPS, *ARGOPECTEN IRRADIANS IRRADIANS*, FED VARYING TETRASELMIS CHUI CONCENTRATIONS AND ITS EFFECT ON AMMONIA CO

James C. Widman Jr. (USDOC, NOAA, National Marine Fisheries Service Milford CT), Shannon Meseck (USDOC, NOAA, National Marine Fisheries Service Milford CT), David J. Veilleux (USDOC, NOAA, National Marine Fisheries Service Milford CT), Mark Dixon (USDOC, NOAA, National Marine Fisheries Service Milford CT)

Bay scallops, *Argopecten irradians irradians*, were grown in semi-static culture systems containing *Tetraselmis chui* at concentrations of 1000, 3500, 8000 and 11000 cells/ml. Our objective was to determine whether phytoplankton concentrations would affect ammonia levels in the culture system. Microalgal cell concentrations were continuously monitored and maintained by a computer control system. F media was used to grow the *T. chui*. Daily buckets were drained and filled with 15L of filtered seawater. Twice weekly scallops were removed from the containers and placed in clean 15L buckets. Every week ammonia levels were measured during a 24 hour period. Scallops grew from initial mean shell heights of 10.5-10.9mm to final mean shell heights of 15.2-17.9mm. Scallops grew at similar rates for the first 14 days. After 21, days scallops grown at a cell concentration of 11000 cells/ml were significantly smaller than those grown at 1000 cells/ml. Throughout the experiment scallops grown at 1,000 and 3,500 cells/ml were not significantly different ($p > 0.05$) from one another. Similarly scallops grown at 3,500 and 8,000 cells/ml grew at similar rates ($p > 0.05$). Scallops grown at 8,000 and 11,000 cells/ml were always similar ($p > 0.05$) to one another. These results contrast with a previous study that had shown no growth differences when scallops were fed varying concentrations of *T. chui* grown in E media.

Ammonia concentrations were never measured above 10umol/L. There was no relationship between ammonia concentrations observed and scallop growth rates. Scallop survival was greater than 83% in all treatments.

OYSTER AQUACULTURE MAY POSITIVELY AFFECT EELGRASS (*ZOSTERA MARINA L.*) THROUGH ENHANCED SEED PRODUCTION AND GERMINATION.

Lorena M. Wisheart (Oregon State University), Sally D. Hacker (Oregon State University), Brett R. Dumbauld (USDA/ARS), Jennifer L. Ruesink (University of Washington)

The interactions between oyster aquaculture and eelgrass need to be explored to assist shellfish growers in the development of sustainable farms while ensuring ecological integrity in aquaculture areas. Past studies have identified both positive and negative effects of shellfish aquaculture on eelgrass but researchers have yet to address how such activities may affect eelgrass recruitment. We conducted surveys in Willapa Bay, WA and found higher seedling densities in dredged beds than in longlines or eelgrass beds. We hypothesized that this pattern was due to variations in seed density and/or differences in germination.

We estimated seed density by counting the number of seeds produced per shoot in each habitat type and found this to be highest in dredged beds and lowest in longlines. We also tested the hypothesis that dredging positively influences germination by adding seeds to each of the three habitat types in areas with eelgrass present and adjacent plots where adult plants were removed. Germination was highest in the eelgrass beds, where, interestingly, eelgrass

removal had a positive effect. We found a similar result when seeds were added to control and removal plots at five different eelgrass beds throughout the bay. Higher germination in removal plots suggests that reduced competition for light and other resources may positively influence recruitment.

Greater recruitment in dredged beds may thus be due to both enhanced seed densities as well as removal of neighboring adults. Together these studies suggest ground culture practices may positively affect eelgrass recruitment while longlines may have a negative effect.

GENETIC DIFFERENTIATION AMONG FOUR CRASSOSTREA ARIAKENSIS POPULATIONS IN ASIA BY MICROSATELLITE POLYMORPHISM

Jie Xiao (Virginia Institute of Marine Science), Jan F. Cordes (Virginia Institute of Marine Science), Kimberly S. Reece (Virginia Institute of Marine Science)

Crassostrea ariakensis is being considered for introduction into Chesapeake Bay to help revive the declining native oyster industry and bolster the local ecosystem. Little is known, however, about wild populations of *C. ariakensis* in its native region, including native distributions, overall genetic diversity, genetic structure, and levels of gene flow. Several discontinuous natural populations have been identified along a wide geographic range in the western Pacific. It's still unclear whether these all are natural populations or whether some are a result of anthropogenic transportations. We studied the genetic variance among 4 wild populations of *C. ariakensis* from Ariake Bay, Japan, Kahwa River, South Korea, the Yellow River basin in northern China, and Beihai in southern China, using novel microsatellite makers. Initial results are reported for 3 loci (CarG110, Car119-6a, Car11-70) developed from *C. ariakensis* partial genomic libraries and screened for the presence of null alleles in 8 family crosses. Estimated multilocus *Fst* (0.0168) values were highly significant for all samples, indicating heterogeneous populations exist in these regions. Single locus *Fst* and *P* values are quite variable among these loci, and additional markers are being developed to further test the null hypothesis of population homogeneity. These preliminary results indicate genetic structure exists among populations of *C. ariakensis* in its native region and suggest that microsatellite markers could serve as efficient genetic tags for monitoring *C. ariakensis* introduced into the Chesapeake Bay from different native gene pools.

GENETIC VARIATION IN SURVIVAL AND POST-STARVATION GROWTH RECOVERY OF BIVALVE LARVAE (CRASSOSTREA GIGAS)

Pauline C. Yu (University of Southern California), Donal T. Manahan (University of Southern California)

Defining the "point of no return" is of importance for larvae growing in nutrient-poor environments. We tested different larval families of *C. gigas* for their starvation tolerance and ability to recover once fed, following prolonged starvation. Larvae were capable of surviving for over 6 weeks without food, representing a >6-fold increase over theoretical limitations based on utilization of energy reserves in eggs. Such larvae grew upon feeding at rates comparable to sibling larvae fed at day 2. Under our experimental conditions, the "point of no return" for some fraction of a larval cohort far exceeded theoretical expectations. Larvae did not down-regulate metabolism, as respiration rates were maintained at ~12 pmol larva⁻¹ hr⁻¹ throughout the period tested for food deprivation. Also, protein content of larvae was conserved in the absence of food with up to 47% of day-2 content being present even after 24 days. Genotypic differences in larval survival and recovery were observed. Eggs from single females showed 2-fold differences in starvation resistance when fertilized with different males, implying important genetic-physiological interactions regarding use of egg energy reserves. When comparing sibling larvae from multiple genetic crosses, size at day 6 for larvae fed from day 2 ("growth heterosis"?) correlated positively (*P*=0.02) with time to 50% survivorship for larvae of the same family that were unfed from day 2. We propose that common mechanisms of physiological efficiency might be the bases for growth heterosis under high-food conditions and increased larval survival in the absence of food.

EFFECTS OF FLUID SHEAR ON RED ABALONE SPERM-EGG INTERACTIONS

Richard Zimmer (UCLA), Jeffrey A. Riffell (UCLA)



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Fertilization rate is an important factor mediating the production of offspring and, hence, population dynamics. For marine organisms that broadcast their sperm and eggs into the sea, surprisingly little is known about the mechanisms controlling sperm-egg interactions. Here, we investigated hydrodynamic effects on fertilization in red abalone (*Haliotis rufescens*). Experiments were performed in laminar-shear flows that simulated important aspects of small-scale turbulence within natural habitats. Fertilization rates were determined over a wide range of gamete concentrations and shears created in a Taylor-Couette flow tank and using a new application of infrared laser and computer-assisted video imaging technologies. For red abalone, shears of $< 0.1/s$ maximized sperm chemotaxis, gamete encounter rates, and fertilization success. Fertilization was subsequently well explained by numerical models that took into account cell shapes, sizes, and propulsive forces generated by sperm swimming, as well as shear forces imposed by the flow. These models thus established the relative contributions of active sperm behavior and passive physical transport to sexual reproductive processes. Combined findings have value in forecasting field sites that maximize offspring production, for transplants, or outplants, of adult brood stock.